Bromo Conference Symposium on Natural Product Biodiversity
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Assalamu’alaikum warahmatullahi wabarakatuh
Good morning
Guest honors
Minister of Research, Technology and Higher Education Republic of Indonesia,
Dean of Faculty of Pharmacy, Universitas Airlangga,
Rector of Universitas Airlangga,
Distinguished speakers,
Participants,
Ladies and gentlemen,

On behalf of the Bromo Conference Committee, I would like to extend a very warm welcome to our distinguished guest in our beloved country Indonesia, to participate in the Symposium on Natural Product and Biodiversity 2018. This conference are collaboration between Universitas Airlangga, The Indonesian Association of Natural Drug Researchers (PERHIPBA), Institute of Tropical Diseases (ITD) and Phytochemical Society of Asia (PSA). I would also like to thank you all for being here, particularly to recognize the time effort in order to present the various organization and be with us today to cover large number of subjects that will have impacts on the role of natural product research in drug discovery.

I believe within this special symposium will become a memorable event for all participants because of its outstanding gathering of the world leader in natural medicine research, legal expertise, as well as many young scientists from diverse fields in order to discuss about the utilization of science and technology to enhance the role of traditional medicine in the health care system

For the successful conference during the next two days, the Bromo Conference Committee did our best to build up a sophisticated scientific program, and to provide all of you the best schedules. We surely hope this important conference will be enjoyable for all participants to learn and share new update of scientific information

Finally, allow me to send our gratitude for the participations of our colleagues from United State of America, Australia, Switzerland, Japan, Philippines, Thailand, and also Malaysia, without their participations, this conference would not be able to be held.

Once again, welcome to Bromo Conference. Wishing you a pleasant and wonderful stay in Surabaya.

Sincerely yours,
Prof. Dr. Bambang Prajogo E.W, MS, Apt
Assalamu'alaikum warahmatullahi wabarakatuh

Indonesian Minister of Research, Technology and Higher Education
Rector Universitas Airlangga,
Our Distinguished Speakers,
Head of Tropical Disease Center,
Head of the Indonesian Association of Natural Drug Researchers (PERHIPBA) and
All Participants.

Ladies and Gentlemen,

Welcome to all distinguished guests, speakers and participants of the Bromo Conference in Surabaya. Surabaya is the second biggest city of Indonesian after Jakarta. This Place Garuda Mukti Room is our special room at Universitas Airlangga for International & National Ceremony.

This Bromo Conference held by Faculty of Pharmacy Universitas Airlangga has the goal to disseminate researches involve the medicinal plants. It is expected that gradually health professionals will be able to help people in the community to stay healthy as prevention as well as medication. Through the Bromo Conference, we hope all researchers from all over the world to enhance mutual collaborations among us. This is the best way to work hand in hand to solve some global health issues. I believe that through improving health standard, we will achieve healthy and better quality of life.

Ladies and Gentlemen,

I would like to extend my gratitude to the organizing committee for their hardwork in making this event a success after the hectic times in the last couple of months.

Finally, I wish all of you a successful event, and enjoy your stay in Surabaya. May this event be memorable and fruitful for all of us.

Thank you very much
Wassalamu’alaikum warahmatullahi wabarakatuh

With my best wishes,
Dr Umi Athiyah, MS, Apt.
Assalamu’alaikumwarahmatullahiwarahmatuh
Good morning
Guest of Honor,
Indonesian Minister for Research, Technology and Higher Education
Distinguished speakers,
Colleagues,

Ladies and gentlemen,

Let us praise Allah SWT the almighty for His blessings for all of us today.
It is a privilege for me to welcome our guests of honor or their representatives, distinguished speakers, participants of the Bromo international conference. This conference is important for its focus on natural products and biodiversity, which is a special wealth of Indonesia. Indonesia is blessed with a huge range of biodiversity, including its medicinal plants. Therefore, research topics and schemes on medicinal plants are on tops of our priorities in Universitas Airlangga. For example, our Institute of Tropical Disease is gearing up its research toward traditional medicinal plants which have been used in different traditional communities for preventing and curing infectious diseases, such as malaria. They also start to uncover the use of medicinal plant for other global diseases such as HIV AIDS.

In this borderless era, I have a strong belief that science is boundary less. The time has thus come for us to collaborate to deal with global issues. Research collaborations, I believe, play a very important role. Both challenges and opportunities have become global. Therefore, our research activities and services have critical economic, societal and environmental impacts. Our research should bring progress, in economic terms as well as in social justice term. Our research should be impactful, strong, and meaningful to overcome local, national, regional and international problems. It should be able to contribute to the betterment of our quality of life.

Ladies and gentlemen,

By holding this conference, I wish to encourage all of us to make a prompt and real action through enhancing our collaborations. Higher education has transformed into an active place to produce relevant knowledge, to provide solutions to societal problem, and to provide high quality education. Our research should be strong, purposeful, and impactful. Our research should also be collaborative and engaging. Moreover, let us not forget that our research should also benefit our students. Considering the high stake of our research, I wish that this conference will provide a platform for future collaboration and research dissemination.
I wish all of you a successful event and enjoy your stay in Surabaya.

Wassalama’alaikumwarahmatullahiwarahmatuh

With my best wishes,
Prof. Dr. H. Mohammad Nasih, MT., SE., Ak., CMA
# ORGANIZING COMMITTEE

## STEERING COMMITTEE
- Rector Universitas Airlangga
- Dean of Faculty of Pharmacy Universitas Airlangga
- President of Phytochemical Society of Asia
- Head of the Indonesian Association of Natural Drug Researchers (PERHIPBA)
- Director of Institute of Tropical Diseases, Universitas Airlangga

## INTERNATIONAL ADVISORY BOARD

### CHAIRMAN
Prof. Jean Luc Wolfender, Switzerland

### MEMBER
- Prof. Kurt Hostettmann, Switzerland
- Prof. Yoshinori Asakawa, Japan
- Prof. Mary J. Garson, Australia
- Prof. Maribel G. Nonato, The Philippines
- Prof. Nor Hadiani Ismail, Malaysia
- Assoc. Prof. Angela Calderon, The USA

## LOCAL ORGANIZING COMMITTEE

### CHAIRMAN
Prof. Dr. Bambang Prajogo EW, MS., Apt

### Co-Chairman
Dr. Aty Widyawaruyanti, MS., Apt

### Secretary
Suciati, S.Si., M.Phil., PhD., Apt

### Treasurer
- Dr. Idha Kusumawati, MSi., Apt
- Febry Annuryanti Wijaya, S.Farm., MSc., Apt

### Administration
- Rr. Retno Widyowati, S.Si., M.Pharm., PhD., Apt
- Gesnita Nugraheni, MSc., Apt
- Arie Sulistyarini, M.Pharm., Apt
- Adita Ayu Permanasari, S.Si
- Hikmatul Ilmi, S.Si., M.Si

### Scientific Program
- Tutik Sri Wahyuni, S.Si., MSi., PhD., Apt
- Dr. rer. nat. ML. Ardhani Dwi Lestari...
- Myrna Adianti, S.Si., MKes., PhD., Apt
- Chrismawan Ardianto, S.Farm., M.Sc., PhD., Apt
- Dr. Tri Widiandani, S.Si., Sp.FRS., Apt.
- Kholies Amalia Nofianti, S.Farm., M.Sc., Apt
- Lidya Tumewu, S.Farm., M.Farm., Apt

### Event Management
- Neny Purwitasari, S.Farm., M.Sc., Apt
- Drs. Herra Studiawan, MS., Apt
- Dini Retnowati, S.Farm., MSi., Apt
- Edith Frederika Puruhito., SKM., MSc
- M.P. Budyandini D. Pramesti, dr, Mkes, SpAnd
- Dra. Sri Musta’ina, Mkes

### Fundraising
- Prof. Dr. Sukardiman., MS., Apt
- Prof. Dr. Nasronudin, dr., Sp.PD., K-PTI., FINASIM
- Dr. Achmad Fuad Hafid, MS., Apt

### Exhibition
- Andang Miatmoko, S.Farm., M.Sc., PhD., Apt
- Abhimata Paramananda, S.Farm., MSc., Apt
<table>
<thead>
<tr>
<th>Section</th>
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</table>
| Workshop                        | Sofyan Kurniawan, S.Farm.  
Prof. Dr. Gunawan Indrayanto  
Dr. Suwidji Wongso  
Retry Sinansari, S.Farm., M.Farm., Apt  
Ratih, S.Farm., Apt  
Mabluhatus Solehah, S.Farm., Apt  
Farizah Izazi, S.Farm., Apt.       |
| Publication and Documentation    | Dr. Wiwied Ekasari, MSi., Apt  
Prof. Dr. Mangestuti, MS., Apt  
Ni Luh Indrawati., S.Farm., Apt  
Sujarwo  
Deddy Dwi Sutanto, S.Farm       |
| Transportation and Accommodation| Mahardian Rahmadi, S.Si., M.Sc., PhD., Apt  
Lismo  
M. Eko Adi Putra       |
| Cateering                       | Lusiana Arifianti, S.Farm., M.Farm., Apt  
Dra. Rakhmawati., MSi., Apt  
Inasah  
Ida Lukitasari  
Nur Aini, Amd       |
| Facilities and Equipment        | Catur Dian Setiawan, S.Farm., M.Kes., Apt  
Budi Rahardjo, S.Sos  
A. Muthi Andy Suryadi, S.Farm., Apt  
Suparto  
Iwan Martono       |
SOCIAL EVENT

- Gala dinner : 11 July 2018, 7 – 9 pm
- Bromo Tour  : 12 July 2018 (10 pm) – 13 July 2018 (10 am)
# CONFERENCE PROGRAMME

## Day 1 July 11th 2018

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>08.00-08.30</td>
<td>Registration</td>
</tr>
<tr>
<td>08.30-10.00</td>
<td>Opening ceremony</td>
</tr>
<tr>
<td><strong>10.00-10.30</strong></td>
<td><strong>KEYNOTE SESSION 1</strong></td>
</tr>
<tr>
<td>Minister of Research Technology and Higher Education of the Republic of Indonesia</td>
<td></td>
</tr>
<tr>
<td><strong>PLENARY SESSION 1</strong></td>
<td>Room: Garuda Mukti</td>
</tr>
<tr>
<td><strong>Chairman: Prof. Jean-Luc Wolfender</strong></td>
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</tr>
<tr>
<td>10.35-11.05</td>
<td>Prof. Mary J. Garson</td>
</tr>
<tr>
<td></td>
<td>Chemical and Spectroscopic Studies on</td>
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<tr>
<td></td>
<td>Bioactive Oxygenated Norditerpenes from Marine Molluscs</td>
</tr>
<tr>
<td>11.10-11.40</td>
<td>Prof. Maribel G. Nonato</td>
</tr>
<tr>
<td></td>
<td>Recent Studies on the “Virtues” of the Family Pandanaeae</td>
</tr>
<tr>
<td><strong>11.45-12.45</strong></td>
<td><strong>LUNCH BREAK, PRAYER, POSTER, and EXHIBITION</strong></td>
</tr>
<tr>
<td><strong>PLENARY SESSION 2</strong></td>
<td>Chairman: Prof. Nor Hadian Ismail</td>
</tr>
<tr>
<td>13.00-13.30</td>
<td>Assoc. Prof. Kornkanok</td>
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<tr>
<td></td>
<td>Ingkaninan</td>
</tr>
<tr>
<td></td>
<td>From Bench to market : Development of Natural Product from Thai Medicinal Plants</td>
</tr>
<tr>
<td>13.35-14.10</td>
<td>Prof. Katsuyoshi Matsunami</td>
</tr>
<tr>
<td></td>
<td>Phytochemical study on Okinawan Subtropical plants</td>
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<tr>
<td><strong>PARALLEL SESSION 1</strong></td>
<td></td>
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<tr>
<td>14.15-14.50</td>
<td>INVITED SPEAKERS 1 (30 mins presentation for presentation and discussion)</td>
</tr>
<tr>
<td>Invited speaker 1</td>
<td>Invited speaker 2</td>
</tr>
<tr>
<td>Prof. Mangestuti Agil</td>
<td>Prof. Bambang Prajogo EW</td>
</tr>
<tr>
<td>Chairman: Prof. Katsuyoshi Matsunami</td>
<td>Chairman : Dr. Beverly Tucker</td>
</tr>
<tr>
<td>R. 301</td>
<td>R. 300</td>
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<tr>
<td>14.45-15.00</td>
<td><strong>COFFEE BREAK</strong></td>
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## ORAL PARALLEL SESSION 1

<table>
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<th>Time</th>
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<tbody>
<tr>
<td>15.05-17.00</td>
<td>ORAL PRESENTATION 1 (Each presenter was given 10 mins for presentation and discussion)</td>
</tr>
<tr>
<td>R. Garuda Mukti</td>
<td>R.300</td>
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<tr>
<td>Chairman: Prof. Sukardiman</td>
<td>Chairman: Pinus Jumaryatno, Ph.D</td>
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<tr>
<td>17.00-17.40</td>
<td>POSTER PRESENTATION 1 (odd number)</td>
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<tr>
<td>PP 1-103</td>
<td>Room: Poster room R. 300</td>
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Day 2 July 12th 2018

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<tr>
<td>08.00-08.30</td>
<td>Registration</td>
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<tr>
<td>08.30-09.00</td>
<td>KEYNOTE SESSION 2</td>
<td>Prof. Jean-Luc Wolfender: Change of Paradigm in Pharmacognosy - Prioritization of Bioactive Natural Products Using Massive Multi-informational Molecular Networks</td>
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<tr>
<td>09.00-09.15</td>
<td>COFFEE BREAK (Hall Lt 4)</td>
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PLENARY SESSION 3

Chairman: Prof. Mary J. Garson

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
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<tbody>
<tr>
<td>09.20-09.55</td>
<td>Prof. Hak Hotta: Broad-spectrum antiviral that target viral envelope lipid bilayers</td>
<td></td>
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<tr>
<td>10.00-10.30</td>
<td>Prof. Nor Hadiani Ismail: <em>Goniothalamus lanceolatus</em>: an ethnomedicine plant of Serawak</td>
<td></td>
</tr>
<tr>
<td>10.35-11.10</td>
<td>Dr. Pawan K. Agrawal: Some Contributions to Natural Product Research</td>
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</table>

PARALLEL SESSION 1

INVITED SPEAKERS 2 (35 mins presentation and discussion)

<table>
<thead>
<tr>
<th>Invited speaker 4</th>
<th>Invited speaker 5</th>
<th>Invited speaker 6</th>
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<tbody>
<tr>
<td>Prof. Maria Inge Lusida</td>
<td>Dr. David C. Sokal</td>
<td>Dr. Atit Kanti (LIPI)</td>
</tr>
<tr>
<td>Chairman: Prof. M Mangestutti Agil R. 301</td>
<td>Chairman: Prof. Bambang Prajogo EW R. 300</td>
<td>Chairman: Prof. Sukardiman R. 305</td>
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<tbody>
<tr>
<td>11.15-11.45</td>
<td>LUNCH BREAK, PRAYER, POSTER and EXHIBITION VIEWING</td>
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ORAL PARALLEL SESSION 2

ORAL PRESENTATION 3 (Each presenter was given 10 mins for presentation and discussion)

<table>
<thead>
<tr>
<th>OP 37-44</th>
<th>OP 45-52</th>
<th>OP 53-60</th>
<th>OP 61-68</th>
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<tr>
<td>Chairman:</td>
<td>Chairman: Retno Widyowati, Ph.D</td>
<td>Chairman: Dr. Triwidianandl; Neny Purwitasari, MSc</td>
<td>Dr. rer. nat Maria Lucia Ardhani D.L., Mahardian Rahmadi, Ph.D</td>
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<tr>
<td>Suciati, Ph.D</td>
<td>R. 300</td>
<td>R. 301</td>
<td>R. 305</td>
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<td>13.00-14.30</td>
<td>POSTER PRESENTATION 2 (even number)</td>
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<tr>
<td>PP 1-103</td>
<td>Room: Poster room R. 300</td>
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<tbody>
<tr>
<td>15.15-15.30</td>
<td>COFFEE BREAK (HALL Lt. 4)</td>
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PLENARY SESSION 4 (30 mins presentation and discussion)

Chairman: Prof. Maribel G. Nonato

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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>15.35-16.05</td>
<td>Prof. Gunawan Indrayanto: Recent Development for Quality Control Method of Herbal derived Drugs</td>
<td></td>
</tr>
<tr>
<td>16.10-16.40</td>
<td>Assoc. Prof. Angela I. Calderón: Quality and Safety Assessment of Botanical Dietary Supplements</td>
<td></td>
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<tr>
<td>16.45-17.00</td>
<td>BEST ORAL AND POSTER PRESENTATION ANNOUNCEMENT</td>
<td></td>
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<tr>
<td>17.00-17.15</td>
<td>CLOSING CEREMONY and PHOTO SESSION</td>
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## SCHEDULE OF INVITED SPEAKERS

### DAY – 1, 11 July 2018

#### Keynote Session 1

<table>
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<th>No</th>
<th>Time</th>
<th>Name</th>
<th>Title</th>
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<tbody>
<tr>
<td>K-1</td>
<td>10.00-11.30</td>
<td>Minister of Research, Technology and Higher Education of the Republic of Indonesia</td>
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#### Plenary Session 1

**Chairman:** Prof. Jean-Luc Wolfender  
**Room:** Aula Garuda Mukti

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<tbody>
<tr>
<td>P-1</td>
<td>10.35-11.05</td>
<td>Prof. Mary J. Garson</td>
<td>Chemical and Spectroscopic Studies on Bioactive Oxygenated Norditerpenes from Marine Molluscs</td>
</tr>
<tr>
<td>P-2</td>
<td>11.10-11.40</td>
<td>Prof. Maribel G. Nonato</td>
<td>Recent Studies on the “Virtues” of The Family Pandanaceae</td>
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</tbody>
</table>

#### Plenary Session 2

**Chairman:** Prof. Nor Hadiani Ismail  
**Room:** Aula Garuda Mukti

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<th>No</th>
<th>Time</th>
<th>Name</th>
<th>Title</th>
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<tbody>
<tr>
<td>P-3</td>
<td>13.00-13.30</td>
<td>Assc. Prof. Kornkanok Ingkaninan</td>
<td>From Bench to Market: Research and Development of Health Products from Thai Medicinal Plants</td>
</tr>
<tr>
<td>P-4</td>
<td>13.35-14.05</td>
<td>Prof. Katsuyoshi Matsunami</td>
<td>Phytochemical Study on Okinawan Subtropical Plants</td>
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**Keynote Session 2**

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<tbody>
<tr>
<td>K-2</td>
<td>08.30-09.00</td>
<td>Prof. Jean-Luc Wolfender</td>
<td>Change of Paradigm in Pharmacognosy – Prioritization of Bioactive Natural Products Using Massive Multi-Informational Molecular Networks</td>
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**Plenary Session 3**
Chairman: Prof. Mary J. Garson  
Room: Aula Garuda Mukti

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<tbody>
<tr>
<td>P-6</td>
<td>09.20-09.50</td>
<td>Prof. Hak Hotta</td>
<td>Broad-spectrum antivirals that target viral envelope lipid bilayers</td>
</tr>
<tr>
<td>P-7</td>
<td>09.55-10.25</td>
<td>Prof. Nor Hadiani Ismail</td>
<td><em>Goniothalamus lanceolatus</em> Miq., an Ethnomedicinal Plant of Sarawak</td>
</tr>
<tr>
<td>P-8</td>
<td>10.35-11.10</td>
<td>Dr. Pawal K. Agrawal</td>
<td>Some Contributions to Natural Product Research</td>
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</tbody>
</table>

**Plenary Session 4**
Chairman: Prof. Maribel G. Nonato  
Room: Aula Garuda Mukti

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<tbody>
<tr>
<td>P-9</td>
<td>15.35-16.05</td>
<td>Prof. Gunawan Indrayanto</td>
<td>Recent Development for Quality Control Method of Herbal derived Drugs</td>
</tr>
<tr>
<td>P-10</td>
<td>16.10-16.40</td>
<td>Assoc. Prof. Angela I. Calderón</td>
<td>Quality and Safety Assessment of Botanical Dietary Supplements</td>
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### Invited Speaker

#### Day - 1

<table>
<thead>
<tr>
<th>Invited Speaker</th>
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<th>Chairman</th>
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<tbody>
<tr>
<td>IS-3</td>
<td>R.305</td>
<td>11.35-12.10</td>
<td>Pinus Jumaryatno, Ph.D</td>
<td>Exploring Terpenoids of Marine Sponges from South-East Queensland</td>
<td>Assoc. Prof. Kornkanok Ingkaninan</td>
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#### Day - 2

<table>
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<tr>
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<th>Chairman</th>
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<tbody>
<tr>
<td>IS-5</td>
<td>R.300</td>
<td>11.35-12.10</td>
<td>Dr. David C Sokal, MD</td>
<td>The Need for New Male Contraceptives and Clinical Research on Natural Products</td>
<td>Prof. Bambang Prajogo EW, MS. Apt.</td>
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## SCHEDULE OF ORAL PRESENTERS

### DAY 1, 11 JULY 2018

**R. GARUDA MUKTI**

**Chairman : Prof. Dr. Sukardiman, MS., Apt.**

**15.05-17.00**

<table>
<thead>
<tr>
<th>TIME</th>
<th>PRESENTER NUMBER</th>
<th>TITLE</th>
<th>AUTHOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.05 - 15.15</td>
<td>OP-1</td>
<td>Bio-similar Nutraceuticals: Does the commercialization and heavy consumption in the western world will kill the global bio diversity</td>
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**R. 300**

**Chairman : Pinus Jumaryatno, Ph.D**

**15.05 - 17.00**

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### Estrogenic Activity Potency of Four Isolated Compounds from Ethyl Acetate Extract of Sasaladaan (*Peperomia pellucida* [L.] Kunth)

I Gusti Agung Ayu Kartika*, Kyu Hyuck Chung, Jong Hwan Kwak, Catur Riani, Muhamad Insanu, I Ketut Adnyana

### Anti-Inflammatory Activity of Catechins Isolated of *Uncariagambir* ROXB in Albino Rats that Induced with Carrageenan

Muhammad Yanis Musdja*, Nelly Suryani, Pipit Pitriyah

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**Chairman : Dr. Triwidiandani; Neny Purwitasari, MSc.**

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-----PLENARY SPEAKER----
Chemical and Spectroscopic Studies on Bioactive Oxygenated Norditerpenes from Marine Molluscs

Mary J. Garson

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072, Australia

*Presenting author
Email: m.garson@uq.edu.au, Phone: +61733653605

ABSTRACT

Marine molluscs use terpenes derived from their sponge diet to aid in their protection from predators. In this talk, I will describe some of the recent identifications of cytotoxic oxygenated norditerpenes isolated from colourful nudibranchs by our research group and highlight the role of advanced NMR spectroscopy and molecular modelling in the structure determination. Notably, the use of quantum chemical calculations for the prediction of proton and carbon chemical shifts provides a valuable tool that aids complex structure elucidation. Selected examples include: spiroepoxide lactones and splendidalactone from Goniobranchus splendidus, daphnelactone from Goniobranchus daphne, and the cyclopropyl-containing verrielactone from Goniobranchus verrieri. Biosynthetic pathways that may explain the formation of these extensively rearranged terpene metabolites are presented.

Keywords: Nudibranchs, terpenes, NMR, DFT calculations, stereochemistry, palatability
ABSTRACT

The Pandanaceae family comprise five genera namely the Pandanus, the biggest of the group, the Freycinetia, the Sararanga, the Benstonea, and the Martellidendron. The Philippines blessed with rich biodiversity have the four genera except the Martellidendron with about 80% endemism of the genus Pandanus. Most of the phytochemical and biological/pharmacological studies conducted were on the Genus Pandanus. These afforded novel/new structural group of alkaloids and new/known compounds with new/known bioactivities. More recently, the Pandanus research program have considered the genera of Freycinetia and Benstonea. This paper will present recent studies pertaining to discovery of antimicrobial, $\alpha$-glucosidase inhibitors, and antitubercular bioactives from the species of genera Pandanus, Freycinetia, and Benstonea. Chemical profiling by metabolomics studies on the three genera will also be presented.
From Bench to Market: Research and Development of Health Products from Thai Medicinal Plants

Kornkanok Ingkaninan

Bioscreening unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand

Email: k_ingkaninan@yahoo.com, Phone: +66-814817350

ABSTRACT

Background: Biodiversity is a potential source of drugs and bioactive compounds. In many countries like Thailand, people still use herbs and traditional medicines for their health including both treatment and prevention of diseases. The “modern” forms of herbal utilization such as food supplements and cosmetics are in great demands for the markets worldwide.

Objectives: We aimed at translational research of Thai medicinal plants for food supplements and cosmetics and finally transferring technology to private sectors. Two examples of our successful projects are research and development of a food supplement for memory improvement containing Bacopa monnieri Wettst. (Brahmi) extract and hair growth control products containing Curcuma aeruginosa Roxb. extract.

Methods: The extract methods of the plants of interest were developed and standardized by the levels of bioactive markers. Multidisciplinary researches in the areas of quality controls, good agricultural practices (GAP), pharmacology, toxicology, pharmacokinetics, pharmaceutics and clinical studies were conducted.

Results: The extract of the shoots of Brahmi was standardized by five saponin glycosides, namely, bacopaside A3, bacopasaponin C, bacopaside I, II, and X. In the case of C. aeruginosa, a sesquiterpene, germacrone which was responsible for anti-androgen activity of the extract was used as a bioactive marker. The extracts were proved to be effective in vitro and in vivo experiments without any signs of acute and chronic toxicities. The monograph and the GAP of Brahmi were published. A double-blinded randomized controlled trial in 60 elderly volunteers confirmed efficacy and safety of Brahmi extract (300 mg/day) for memory and concentration improvement. As for C. aeruginosa, a double-blinded randomized controlled trial in 87 male pattern baldness volunteers suggested that a hair- tonic containing C. aeruginosa can be a hair re-growth promoter. In addition, the extract was used as an ingredient in a roll-on for axillary hair growth retardation purpose. A randomized controlled study conducted with 30 female volunteers demonstrated that the roll-on containing 1% and 5% of C. aeruginosa extract could reduce the axillary hair growth.

Conclusion: The standardized extracts of Brahmi and C. aeruginosa were developed and tested in vitro, in vivo and in humans. Recently, we have licensed a Brahmi tablet and hair care products containing C. aeruginosa extract to the pharmaceutical and cosmetic companies.

Keywords: antiandrogen, Bacopa monnieri, Curcuma aeruginosa, hair growth, memory improvement, R&D, Thai medicinal plants
P-5

Phytochemical Study on Okinawan Subtropical Plants

Katsuyoshi Matsunami and Hideaki Otsuka

1Department of Pharmacognosy, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima, 734-8553, Japan
2Faculty of Pharmacy, Yasuda Women’s University, 6-13-1 Yasuhigashi, Asaminami-ku, Hiroshima 731-0153, Japan

*Presenting author
Email: matunami@hiroshima-u.ac.jp, Phone: +81-82-257-5335

ABSTRACT

The Okinawa Islands are a crescent-shaped archipelago located south of mainland of Japan. In biogeographically, the distribution boundaries of flora and fauna are known as Watase and Hachisuka lines to the south of Yakushima and Okinawa Island, respectively. Since each island is surrounded by the sea, many plants and animals have evolved there in unique ways for an extremely long period of years. At the present day, thenatural forests of the Okinawa Islands hold a huge variety of unique subtropical plants with relatively high endemism. The taxonomy of Okinawa Islands includes 286 families, 1961 genera, and 5793 plant species, which represents 81.7% of total species (7087) in Japan, although the area of Okinawa is only 0.6% of Japan.1

Recently, a concept of “resource nationalism” spreads through the countries having rich natural resources to realize their ownership and necessity for controlling their precious natural resources. And the Convention on Biological Diversity (CBD) took effect in 1993, with the objectives to conserve the biological diversity, the sustainable use, and the fair and equitable sharing of the benefits arising from the utilization of natural resources. Thus, the scientific investigation and discovery of usefulness of their plant resources are becoming urgent issues to accelerate the conservation of them. In view of this situation, we have focused on the plant resources of our country, especially Okinawan subtropical plants, and have performed phytochemical study on Okinawan subtropical plants for many years. In this symposium, we describe our recent research progress on the isolation of new compounds and their various bioactivities.

Keywords: Okinawa, subtropical plant, glycoside

Reference:
K-2

Change of Paradigm in Pharmacognosy-
Prioritization of Bioactive Natural Products Using Massive Multi-Informational Molecular Networks

Jean-Luc Wolfender¹*, Pierre Marie Allard¹, Emerson Ferreira Queiroz²

¹School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU – Rue Michel Servet 1, 1211 Geneva 11, Switzerland

*Presenting author
Email: jean-luc.wolfender@unige.ch, Phone: +41 22 379 33 85

ABSTRACT

With the recent progresses made in metabolite profiling and bioassays, a question that arises is: do we still need to perform conventional large-scale bioactive guided isolation of natural products? These rapid innovations may lead to a change of paradigm in natural products research. In particular High resolution mass spectrometry (HRMS) and data dependent MS/MS analyses provide very valuable information on secondary metabolites for in-depth metabolome annotation studies.¹ Molecular network (MN) approaches for the mining of such data in combination with spectral database generated in silico² gives the possibility to establish relationships between metabolites thus significantly improving the efficiency of dereplication.³ Such information can be statistically correlated with bioactivity data on extracts and potentially enable the localisation of interesting hits without the need for a bioactivity-guided approach. Ideally a combination all these state-of-the-art methods, using also associated contextualized pharmacognosy metadata,⁴ should enable to identify and localise valuable NP efficiently at the analytical scale. In such a way large scale MS-targeted isolation of valuable NPs only can become a very rational way to conduct investigations. Different recent applications of our metabolomics and phytochemical investigations will illustrated these aspects. An ideal workflow will be presented and discussion on what is readily implemented and is still required will be made.

Keywords: Dereplication, metabolite profiling, metabolomics, MS-targeted isolation

References:
ABSTRACT

Background: Hepatitis C virus (HCV), Dengue virus (DENV) and Japanese encephalitis virus (JEV), which belong to the family Flaviviridae, bud through the membranes of the endoplasmic reticulum (ER), acquiring their envelope lipid bilayers from the ER. The phospholipid content of the ER membranes differs from that of the plasma membrane (PM). Consequently, the phospholipid content of HCV and DENV was reported to differ from that of influenza virus (Orthomyxoviridae), Sendai virus (Paramyxoviridae) and Semliki Forest virus (Togaviridae), which bud through the PM. The phospholipase A₂ (PLA₂) superfamily consists of a large number of members that specifically catalyze the hydrolysis of phospholipids at the sn-2 acyl bond. They show a wide range of sequence variations and functional divergences.

Objective: We aimed to find a particular PLA₂ that targets viral envelope lipid bilayers without damaging the PM of the host cells.

Methods: We tested possible antiviral activities of a number of different PLA₂s obtained from venoms of a snake and honeybee, bacteria and bovine and porcine pancreas against a panel of viruses, which bud through either the ER, ER-Golgi intermediate compartment (ERGIC), trans-Golgi network (TGN) or the PM. Cytotoxicity against the host cells and red blood cells was also evaluated.

Results: We found that the CM-II isoform of secreted PLA₂ obtained from Naja mossambica mossambica snake venom (CM-II-sPLA₂; purchased from Sigma-Aldrich) possessed potent virucidal (neutralizing) activity against HCV, DENV and JEV, with 50% inhibitory concentrations (IC₅₀) being 0.036, 0.31 and 1.34 ng/ml, respectively. On the other hand, the IC₅₀ values of CM-II-sPLA₂ against viruses that bud through the PM (influenza virus, Sendai virus and Sindbis virus [Togaviridae]) or TGN (herpes simplex virus [Herpesviridae]) were >10,000 ng/ml. Moreover, the 50%-cytotoxic concentrations (CC₅₀) of CM-II-sPLA₂ as determined by WST-1, LDH release and hemolysis assays were 17,000, 16,000 and >10,000 ng/ml, respectively, with its selectivity index (CC₅₀/IC₅₀) against HCV and DENV being >30,000. We also found that CM-II-sPLA₂ possessed potent virucidal activity against HBV, which belongs to the family Hepadnaviridae, with an IC₅₀ value being 0.25 ng/ml. The major budding site of HBV particles is still a matter of debate.

Conclusion: Our results suggest that CM-II-sPLA₂ and its derivatives are good candidates for the development of broad-spectrum antiviral drugs that target viral envelope lipid bilayers derived from the ER membrane.

Keywords: Phospholipase A₂, Antivirals, Broad-spectrum, Hepatitis C virus, Dengue virus, Flaviviridae, Viral envelope, Lipid bilayer, Endoplasmic reticulum.


**ABSTRACT**

*Goniothalamus lanceolatus* Miq. is an ethnomedicinal plant indigenous to Sarawak, Malaysia. Parts of this plant are used as traditional remedy by local communities to treat various diseases such as fever, skin infection and been used as mosquito repellent. It is also used by the indigenous people as a treatment for cancer.

We investigated the antiplasmodial activity and acute toxicity of the methanol (MeOH) extracts of the leaves and roots, and the dichloromethane (DCM) extracts of the stem bark, leaves, and roots of *G. lanceolatus*. *In vivo* antiplasmodial activity was assessed using the 4-day suppressive test against *Plasmodium berghei* ANKA (PbANKA) in mice. The plant extracts were administered intraperitoneally (i.p.) as a single dose (30 mg/kg) starting 4 h after infection. At a dose level of 30 mg/kg (i.p.), the DCM extracts of the stem bark and leaves, and the MeOH root extracts, prolonged the survival period of infected mice compared to that of the negative control. The highest level of parasitemia suppression was recorded in mice treated with the DCM stem bark extract at 66.3%. No mortality was observed in mice treated with the DCM extracts of the stem bark and leaves, and the MeOH extract of the leaves, indicating that the LD₅₀ is greater than 300 mg/kg. Both the MeOH and DCM extracts of the roots showed toxic effects at a dose of 300 mg/kg (i.p) with 83.3% mortality rate. The results obtained indicate that the stem bark of *G. lanceolatus* possesses good antiplasmodial activity against PbANKA infected mice without causing acute toxicity. Five known styrylpyrone derivatives namely goniodiol, 8-epi-9-deoxygonioppyrone, 9-deoxygonioppyrone, digoniodiol and goniotalamin were isolated from the bark of *G. lanceolatus*.

On the other hand, at 100 µg/ml concentration, the dichloromethane root extract of *G. lanceolatus* exhibit cytotoxic activity *in vitro* against a panel of human colon and lung cancer cell lines with percent cell viability less than 15%. Two new alkaloids, (−)-goniolanceolactam (1) and 2-acetyl-3-amino-1,4-naphthoquinone (2), along with two known naphthoquinone alkaloids, 2-acetyl-3-amino-5-hydroxy-1,4-naphthoquinone (3) and cleistopholine (4) were isolated from this cytotoxic dichloromethane root extract. The structures were elucidated by spectroscopic techniques and the absolute configuration of 1 was established by single-crystal X-ray diffraction. Alkaloid 1 showed cytotoxic activity on human colon and lung cancer cell lines with IC₅₀ values ranging from 5.32 to 9.91 µM. These findings suggest 1 as a cytotoxic compound against human colon and lung cancer cell lines whose mechanism of action should be explored.

**Keywords**: *G. lanceolatus*, antiplasmodial, cytotoxicity.
Some Contributions to Natural Product Research

Pawan K. Agrawal

Natural Product Inc., 7963, Anderson Park Lane, Westerville, Ohio USA

ABSTRACT

The fascinating field of natural products began with collection of plant, fungal, marine organisms etc., isolation and characterization of chemical constituents, many being found to have utility as medicinal/bioactive compounds, flavor/fragrances and the like. The chemical investigation on Indian medicinal plants (Aesculus indica, Agave cantala, Callicarpa macrophylla, Cedrus deodara, Solanum torvum and Phyllanthus niruri) has led to isolation of several new and many known compounds. The aim of this presentation is to share “highlights from the past” and our contributions by introducing “Natural Product Communications (NPC)” to serve community of natural product researchers.
ABSTRACT

Pharmaceutical industries should apply a rigorous QC (quality control) for ensuring the consistency, safety, and efficacy of their herbal derived drug-preparation. QC must be performed on every stage of production i.e. incoming raw materials, extractions, in-process-control, finished-products and keeping-samples. Due to the complexity nature of the herbal drugs, two approaches of QC should be carried out, that is quantitative determination of the selected marker(s) compound(s), and metabolite profiling. Contamination of herbal medicines with heavy metals, pesticides, toxic metabolites, microbial toxins, pathogenic microorganisms and other foreign matters should be also evaluated.

Combination of chemical profiling and multivariate analysis (MVA) is recommended as the QC tool for botanical identification method (BIM) of herbs, extracts, herb materials, and herb-drug-preparations. Microscopically method or DNA profiling or chemical marker(s) are not recommended to be used as sole BIM due to the lack of specificity. Only marker(s) that have certain criteria’s (Q-marker or MaRS ranking >3) can be utilized as a QC tool. The limit specification range of marker, which used as QC tool, should be described in its analytical target profile (ATP)

For having reliable results of any analysis that have been performed at any QC laboratory, the analysis method must be validated according to newest guidance’s. Sample detection limit of any chemical toxic compound(s) should be lower than its cut-off value and MPL. The reliability of any results of analysis of QC laboratory must be evaluated by using QC-samples for each series of measurements.

Keywords: Chemical-profiling, DNA-profiling, herbal drugs, MaRS ranking system, metabolite profiling, multivariate analysis, sample detection limit, Q-marker, validation method
ABSTRACT

**Background:** Total retail sales of botanical dietary supplements in the United States increased by an estimated 6.8% in 2014 marking the 11th consecutive year of growth. The increase of the sales relies on the rising popularity of the use of botanical dietary supplements in the US among the adults in the last 10 years. Since botanical dietary supplements are increasingly consumed world-wide, calls for global quality and safety standards are emerging.

**Objective:** In order to ensure the good quality and safety of botanical dietary supplements, the following steps should be properly addressed: 1) acquire and authenticate natural product material using good agricultural and good collection practices, 2) determine mechanisms of action and identify active compounds, 3) chemical and biological standardization, 4) absorption, metabolism, and bioavailability of active compounds, 5) preclinical assays of inhibition and induction of drug metabolizing enzymes and transporters, 6) GMP preparation of standardized formulation, and 7) preclinical animal studies/clinical investigations.

**Methods:** Mass spectrometry (MS) and hyphenated techniques such as liquid chromatography–mass spectrometry (LC–MS) have become primary tools in these endeavors.

**Results:** In the last nine years, my research group has been developing and applying methods based on the qualitative and quantitative use of high resolution mass spectrometry to a wide variety of the botanicals and formulations (*Justicia species*, *Euterpe oleracea* Mart (açaí), *Lepidium meyenii* Walpers (maca), East Indian Sandal oil, *Thymus vulgaris* essential oil, and eighteen plants containing YANG XIN® traditional Chinese medicine (TCM) formulation). Specific examples of in-house applications of the mass spectrometry (LC/MS and LC/MS/MS) using the listed botanicals will be discussed in detail.

**Conclusion:** Chemical and biological standardization would help to improve the reproducibility of botanical dietary supplement preparations, while additional ADME studies and clinical investigations of drug-botanical interactions would enhance our understanding of the safe use of these products.

**Keywords:** botanical dietary supplements, quality, safety, LC–MS.
-----INVITED SPEAKERS-----
Neuroprotective Effect of Medicinal Herbs in Indonesian Traditional and Folk Medicines for Women

Mangestuti Agil*

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University

*Presenting author
Email: mmangestuti@yahoo.com, mangestuti@ff.unair.ac.id

ABSTRACT

Indonesia is endowed with abundant medicinal plant resources and traditional medicinal practices. As with other traditional system of medicines, Indonesian women have been engaged in using various mixtures of herbs throughout their life cycle. A pattern of using it is the same among more than 300 ethnic groups of Indonesia, mainly certain formulas for adolescence, adulthood and old ages. Generally, each formula consists of herbs which empirically have been proven of their health properties. Various rhizomes of Zingiberaceae family, for example, are popular, where many formulas contain more than one kind of the rhizomes. Many spices and herbs may present, including clove, mace, pepper, nutmeg, cinnamon, cardamom, fenugreek, fennel, dill, and coriander. Most of the herbs have already been investigated of their health benefits for vital organs in the human body, and therefore is important to prevent diseases caused by many factors, including disease of the nervous, circulatory, respiratory, digestive, and genito-urinary systems. Disease of the nervous system is one of the health problems faced by women when they reach menopause as their final cycle of life. The main factor contributes to it is the lowering of blood estrogen level. Estrogen plays a pivotal role in the overall health, including the nervous system, and therefore, decline of its blood concentration may cause some health problems related to the function of the nervous system, such as dementia. There is a vast growing number of postmenopausal women suffered from Alzheimer, the most common type of dementia. As is indicated through some studies, the regular use of medicinal plants as part of the tradition in Indonesia proved to be able to maintain the health status of women. This may open the possibility of a reduction in the risk of neurodegenerative disorders. Some herbs which have been studied for their activities in the nervous system are those with antioxidative properties, herbs contain phytoestrogenic compounds, and terpenes of essential oils. Plants are potential sources of natural antioxidants proved to be essential in the inhibition of the reactive oxygen species necessary for the survival of living organisms. It is therefore, the development of cognitive enhancers from plants possessing antioxidants has gained much attention due to the role of oxidative stress-induced cognitive impairment. The antioxidant activity is caused by the presence of polyphenols in some plants belonging to the Zingiberaceae family, mace, cardamom, clove, etc. Curcumin, a potent antioxidant of turmeric, has been studied of its neuroprotective activity. Ginger has also been studied of its in vitro neuroprotective property. Mixtures of aromatic herbs have been used in the traditional medicine system of Indonesia. Some formulas prepared from a variety of aromatic herbs have been used for different purposes, mostly for cosmetic use, including special powders for postpartum care, skin scrubs, masks, and soaps. The application of such mixtures as incense for body care is very popular. Scientific research has proved the physiological effects of inhaling the essential oil contents of the aromatic herbs. Terpenes are the main components of essential oil, and research showed the activities of some terpenes as anti-inflammatory, anti-tumor, and neuroprotective effects against neurodegenerative. Study on the monoterpenes, cineole and α-pinene showed their antioxidant activities against reactive oxygen species, and this is the mechanism of antioxidant against neurodegenerative diseases. Eventhough only a few studies have been conducted to understand the effects of terpenes on neuronal health, there still lies a big optimistic to find more that may support their future therapeutic application through regular uses of the herbs.
Phytoestrogens are plant components that have been studied for their contribution in the maintenance of cognitive health among menopausal women. Isoflavone and lignan are estrogen-like compounds occurring naturally in plant foods especially soy. Recent trials showed the activity of soy isoflavone in improving cognitive function among students and post-menopausal women. Research indicated the effect of isoflavones on the brain function through estrogen-independent mechanisms, but studies still have to be done to understand fully about its role in the brain.

*Marsilea crenata*, the local name of semanggi, is a popular aquatic plant of East Java Province of Indonesia. Leaves of the plant have been used empirically to prevent osteoporosis among postmenopausal women. Research found estrogen-like compounds in the leaves which showed their anti-osteoporosis activity in laboratory mice. Preliminary research to study the activity of leaf extracts against neurodegeneration in estrogen-deficient mice showed promising results.

It is concluded, that medicinal herbs in Indonesian traditional and folk medicines are good sources of active ingredients able to protect women from neurodegeneration. Such protection is needed to prevent them from further loss of memory which deteriorate their quality of life. Further scientific research is necessary in giving enough support to the application of medicinal herbs among women as one of the important ways in enhancing their wealth throughout their lives.
ABSTRACT

The information of researchers (1987), which had done the survey in back country of Papua about Traditional Medicine (OT), they had found some ingredients of traditional medicine, which had been using by local inhabitant. One of the interesting points is related to the male and female reproduction, either to increase fertility or contraception. When they were visiting traditional market in Sentani, they found jamu sellers, who sell gendarusa leaves to decrease the birth rate or as male contraception. From the information that we obtained, if we stem the whole plants, then we drink those water, it will make unfruitfulness for the males. In the tradition of that area, there is a relation between a man who wants to marry a girl and he can not afford the dowry, but they still can get married; or they will live in a house, but they are not supposed to have a child before he pays the dowry. There is something interesting here from the consuming of the plant, which is come from Papua, is consumed by drinking the stem of the whole gendarussa plant 30 minutes before using it. It brings the own traction to reveal the truth and until now, we are still doing a consistent and deep research to achieve the product.

Gendarusa plant, which had found and grown in Papua traditionally, has been consuming as male contraception. As a base of knowing deeply to prove that there is a correlation between activities and compound content, it needs the plant’s validity (authentic) with the help of pharmacognosy study and phytochemical study. In the first study, those plants can grow at the entire area of Indonesia, even South East Asia and South Asia. According to literature trace, it has been known that, this plant has a scientific name called Justicia gendarussa Burm. f. (Acanthaceae), appropriate to morphology feature, which has shape of bilabiate flower, single lancet leaf face each other with the same color and purple specification on the leaf bone, stem, and branch as clump. Considering this plant could be grow in the entire Indonesia, so the researcher chooses several plants, which can grow in Surabaya area and other area in East Java to experimental study. According to the field observation, broad area with direct sunbeam and water irrigation are good habitat and it shows the good growth by its green leaves. Those places are field, riparian, and fence plants. For the overall research planning, it needs the selected area to stock continuity, and plants’ information are also used as muscle and joint pain and big supply from Mojokerto for market and cultivated to research material in agriculture area.

The study in Phytochemical area is the first detection in 1986, it stated that gendarussa leaves contained as the class of glycoside flavonoid without knowing kind of bound sugar, besides there was no complete publication. In 1995, the researcher was using LC-MS, which had 12 flavonoid compounds and had 535 molecule weights so that compounds would be having isomer and it was interesting to become an identity. The using of MPLC preparative in 2001 only produced two isolates with proton elucidation and carbon structure analysis showed apecgenin derivation which were bound in xylose and arabinose carbohydrate, would be named as gendarusin A, and B. Both of the compounds were just found in gendarusa leaves even it was found in other plants. In 2011, it produced three gendarusin compounds such as C, D, and E and also some alkaloids. At this moment, metabolite and protein profile studies has been working in several different area to support the tracing of land condition, which is appropriate with the highest active compounds content. Gendarusin A is used by marker compound to the purpose of male antifertility activities test, which had already done, has been doing, and will be done by researcher.

Keywords: Justicia gendarussa Burm.f., gendarusin, male contraception
Exploring Terpenoids of Marine Sponges from South-East Queensland

Pinus Jumaryatno¹*, Joanne T. Blanchfield², Mary J. Garson²

¹Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Yogyakarta 55584
²School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072, Australia

*Presenting author
Email: pinus.jumaryatno@uii.ac.id, Phone: +62274895920

ABSTRACT

Background: Marine sponges are the rich sources of unique secondary metabolites including terpenoids. As part of chemical studies of marine sponges from South-East Queensland, two sponge specimens namely Acanthella cavernosa and Dysidea sp. were investigated.

Objective: To study the chemical composition of marine sponges collected from Mooloolaba, South-East Queensland.

Methods: Sponges were extracted using DCM/MeOH (1:1) followed by partitioned between EtOAc and H₂O. The crude EtOAc extracts were subjected to flash column chromatography using a solvent gradient polarity and then purified by NP HPLC. The structure of isolated compounds was characterised using spectroscopic methods including 1D and 2D NMR, GC/MS, MS and by comparison with literature data.

Results: The Acanthella cavernosa yielded 17 compounds including a rare isocyanate compound, axisocyanate-3, a novel 10-isothiocyanatoguai-6-ene, axisothiocyanate-2, and a pair of bisabolene-type sesquiterpenoids. Meanwhile, a new chromodorane diterpene, chromodorolide E, was isolated from Dysidea sp. together with a series of the known chromodorolides A, B, C, D and a norrisolide-type compound, norrandin.

Conclusion: A various structure types of terpenoids was displayed from the isolated metabolites of Acanthella cavernosa and Dysidea sp.

Keywords: Acanthella cavernosa, Dysidea sp., sesquiterpenoids, chromodorane diterpene.
Hepatitis B and C Virus Infection in Indonesia and its Genetic Diversity

Maria Inge Lusida

Institute of Tropical Disease, Universitas Airlangga, Campus C Unair, Surabaya 60115, Indonesia

*Presenting author
Email: ingelusida@itd.unair.ac.id; Phone: +62-31-5992446

ABSTRACT

Indonesia experienced intermediate to high hepatitis B virus (HBV) endemicity. Before adoption of universal infant vaccination, the prevalence of hepatitis B surface antigen (HBsAg) seropositivity was estimated to be 5–10% in the general population. The risk for chronic HBV infection is highest among those infected during infancy. Fifteen years after adoption of the program, the seropositive rate of HBsAg in preschool- and school-aged children ranged from 2.1% to 4.2% and 0% to 5.9%. The low birth dose coverage and the presence of a vaccine-escape mutant might contribute to the endemicity of HBV infection among children in Indonesia. Other focus areas that will be discussed would be on Occult hepatitis B infection (OBI), HBV infection among high-risk patients, such as hemodialysis patients and HIV community in Indonesia.

Genome-wide association studies have shown that polymorphisms in Interleukin-28B (IL-28B) is associated with Response of Chronic Hepatitis C Patients to Pegylated Interferon/ Ribavirin. The study among Indonesian patients demonstrated that mostly were infected with hepatitis C virus (HCV) genotype1; however, they carried mainly the major genotypes of IL-28B single nucleotide polymorphisms (SNPs), and they mostly achieved sustained virological responses to pegylated interferon/ ribavirin treatment. Although powerful antiviral drugs have been developed recently against HCV, their extremely high costs are far beyond the reach of the vast majority of people living with HCV infection. Development of complementary and/or alternative drugs for treatment of HCV infection is still needed from clinical and economic points of view. Antiviral substances obtained from medicinal plants are potentially good targets to study. A variety of plants have been tested and proven to be beneficial as antiviral drug candidates against HCV. HBV and or HCV infection related studies are still interesting in Indonesia.

Keywords: Hepatitis B, hepatitis C, Indonesia.
The Need for New Male Contraceptives and Clinical Research on Natural Products

David C. Sokal, MD1,2 and Beverly Tucker, MPH, MPIA1*

1 Male Contraceptive Initiative, Durham, NC, USA; 2 Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA

*Presenting author
Email: beverly@malecontraceptive.org, Phone: +1-919-280-1249

ABSTRACT

Background: Despite decades of contraceptive research and development, men have only two modern methods, vasectomy and condoms. Herbal products are being developed as male contraceptives, including Gendarussa at the University of Airlangga.

Objective: Describe a framework for clinical research and registration of herbal products for male contraception.

Methods: Review male contraceptive research, clinical trial methods for contraceptives, and patent and regulatory considerations.

Results: At least two herbal products are under investigation as potential male contraceptives. Registration rules for pharmaceuticals are similar across countries due to the International Council on Harmonisation (ICH); the ICH does not include herbal products and the rules for herbal products differ greatly. For contraceptives, clinical outcomes are clear: pregnancy or no pregnancy. We suggest doing clinical trials of effectiveness for herbal, male contraceptives, and regulatory agencies may require such research. One possible clinical trial design is to recruit couples who desire children and are willing to delay a pregnancy. In such couples, it is ethical to randomize them to drug or placebo. Patent protection for natural compounds is currently uncertain in the United States, increasing the importance of trade secrets. In the US, even without patent protection, a company may obtain five years of market exclusivity from the US FDA, by taking an herbal product through the pharmaceutical approval process. Male Contraceptive Initiative (MCI), a US-based nonprofit, is helping researchers prepare for that process.

Conclusion: Registration of an herbal product for male contraception is likely to require rigorous research, like that required for pharmaceuticals.

Keywords: Male contraception, reproductive biology, natural products, family planning
DAY 1-PARAREL ROOM 1
(Garuda Mukti)-
(OP 1-OP 9)
Bio-similar Nutraceuticals: Does the commercialization and heavy consumption in the western world will kill the global bio diversity

Yashwant Pathak

Faculty Affairs, College of Pharmacy, University of South Florida Health, 12901 Bruce B Downs Blvd, MDC 030, Tampa, FL 33612.

*Presenting author
Email: ypathak1@health.usf.edu, Tel: 813-974-1026

ABSTRACT

Bio-similar Nutraceuticals: The Nutraceuticals market is growing significantly. They are forecasting about $176 Billion market size by 2020. The significant increase in the market size is creating pressure on the naturally available ingredients used in Nutraceuticals. Hence, people are exploring possibility of using genetically modified materials to be used in place of natural materials. No one has ever studied or reported about the usefulness of this material since these were not available in the early times. This is more challenging in the traditional medicine systems such as Chinese medicine and Ayurvedic medicines. These products, many of which are based on natural plants and have lot of traditional processes before the product reaches the patients. With the advent of genetically modified natural products will they exactly behave the way the natural products are behaving or different. When you use these genetically modified materials in Nutraceuticals, herbal drugs or natural products are you really comparing apples with apples or you are comparing apples with oranges. Another challenge for Bio similar s in this field is heavy consumption of these products in the western world and that is virtually depleting the natural resources and also killing the biodiversity globally. Many of the herbal species may be extinct in near future due to this pressure. This presentation will cover the challenges for such products and a need for global standardization process for Nutraceuticals and their ingredients as well as policies to protect the biodiversity.
Development of New Methods for the Synthesis of Dihydropyrans (DHP) and Tetrahydropyrans (THP)

Nadiah Mad Nasir*, Dr Paul Clarke

Department of Chemistry
Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia
nadiahmadnasir@upm.edu.my, +60198501706

ABSTRACT

Recently the group has focused on the development of new methods, base on the Maitland-Japp reaction, for the formation of dihydropyrans (DHP) and tetrahydropyrans (THP), which are structural units found in many biologically active natural products. Extension of the Maitland-Japp reaction now allows for the synthesis of DHPs which can be converted into highly functionalised THPs found in natural products like Lasonolide A (1). The 2, 6-cis-THP A-ring of 1 can be produced from a DHP by conjugate reduction and trapping of the resulting enolate. The 2, 6-trans-THP ring of Diospongin B 2 can be produced from DHP by conjugate addition. The development of this reaction and its scope and limitations will be discussed further.

Keywords: Organic Synthesis, Tetrahydropryan
The Pattern of Complementary Alternative Treatment Used by Cancer Patients

Mochamad Djunaedi¹*, Wahyu Utami¹, Anila Impian Sukorinï

¹ Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia

*Presenting author
Email: Mochamad-d@ff.unair.ac.id, Phone: +6282132654068

ABSTRACT

**Background**: Complementary alternative treatment used among cancer patients is expectedly supporting the achievement of the goal of therapeutic.

**Objective**: This study aims to identify the attitudes of cancer patients related to the use of herbs, supplements or traditional medicine as an alternative complementary medicine.

**Methods**: A cross-sectional study is used in data retrieval using a "semi-structured questionnaire" in 2016 against 100 respondents. Only 75 are eligible and meet the inclusion criteria.

**Results**: Recommendations for the use of alternative complementary medicine are found to originate from other than the health professionals (85%). The use of herbs, traditional medicines or supplements done without prior assessment (77%). Patients use complementary alternatives when the condition of the disease has spread (87%).

**Conclusion**: Integration of health personnel, including pharmacists to assist the team in using herbs, supplements or traditional medicine has not been done optimally.

**Keywords**: Complementary Alternative Treatment, Cancer, Health Professional
The Utilization of Daun Wungu Ethanol Extract (*Graptophyllum pictum (L) Griff.*) As The Endometrial Hyperplasia Inhibitor of Ovariectomy Mice

Listijani Suhargo* and Dwi Winarni

*Department of Biology, FST, Universitas Airlangga, Surabaya 60115, Indonesia

*Presenting author
Email: lis.suhargo@gmail.com, Phone: +6281235442190

ABSTRACT

Endometrial hyperplasia was caused as a side effect of estradiol hormone using for menopausal condition and it could change to be cancer. Daun wungu ethanol extract with its flavonoid competes with estradiol to bind the estrogen reseptor and decreases the side effect of estradiol. The aim of this research was conducted to evaluate the utilization of daun wungu ethanol extracts as the endometrial hyperplasia inhibitor of ovariectomy mice. This research used 30 ovariectomized mice. The ovariectomized mice were grouped in 5 groups for K1 (the control group), P1, P2, P3 and P4 (the treatment with daun wungu extracts 0.5 mg, 0.75 mg, 1 mg and 1.25 mg in 0.1 ml coconut oil). And the all groups were also given 0.06 ug ethinyl estradiol orally and daily. All treatments were done for 30 days. At the end of the treatments, all mice were sacrificed and were made histology preparation of uterus. The result showed that daun wungu ethanol extracts could inhibit endometrium hyperplasia that were showed in decreasing uterus diameter, the thickness of epithelial lining and the abnormal gland percentage. And 0.75 mg daun wungu ethanol extract was optimal conentration as inhibitor for endometrium hyperplasia.

Keywords: daun wungu extracts, endometrium, ovariectomized
ABSTRACT

Background: Talang Mamak is one of the tribes in Province of Riau and the people use plants for many purposes such as food and cloth. Not only is the people use plants for these purposes, plants are one of the important things for their survival. They use plants as medicine to treat many diseases such as diabetic mellitus.

Objective: In this study, we explored some medicinal plants from Talang Mamak tribe as antidiabetic agents.

Methods: Ten medicinal plants were extracted using maseration followed by ultrasonication. The ethanol extracts were analyzed for their antidiabetic activity by using in vitro α-glucosidase inhibitors method.

Results: The results showed that seven species exhibited various antidiabetic activity with IC₅₀ between 41.23-93.34 µg/mL. Flacourtiarukam showed the highest activity with IC₅₀ 41.23 µg/mL followed by Lithocarpusbancanus (47.65), Antidesmabunius (67.33), Macarangabancana (45.58), Macarangagigantea (56.72), Anisophylleadisticha (80.67), and Coleus scutellaroides (93.34).

Conclusion: In this study, it was shown that some medicinal plants from Talang Mamak possessed antidiabetic activity.

Keywords: Talang Mamak tribe, α-glucosidase inhibitors, antidiabetic agents.
ABSTRACT

Background: Different civilizations have used plants as antimicrobial agents to combat different bacteria, fungi, and viruses. Talang Mamak, a Proto Malay tribe in Province of Riau, has been using the plants to treat various infections. However, the antimicrobial activities from these plants have not been reported before.

Objective: In this study, we justify antimicrobial activities from 10 species from Talang Mamak medicinal plants.

Methods: Ten medicinal plants were extracted using maceration followed by ultrasonication. The antimicrobial activities from ethanolic extracts were determined from their growth inhibition by using resazurin method against several pathogenic bacteria and fungal.

Result: The results showed that 70% from the species inhibit the growth of Staphylococcus aureus, 60% against Escherichia coli and 40% against Candida albicans.

Conclusion: In this study showed that some medicinal plants from Talang Mamak possessed antimicrobial activities.

Keywords: Talang Mamak tribe, antimicrobial, resazurin method
**ABSTRACT**

Humans are at a continuous battle against different types of diseases, so that extraordinary effort to accelerate drug discovery has become a necessity. Indonesian biodiversity is abundant natural resources that can be utilized as potential drug sources. Mangroves are among potential plant medicine that grow nearly at all Indonesian coastlines. The aim of this study was to evaluate the potential of mangrove extracts (extract library) as antibacterial agents. In this study, eight mangroves species were used. There were 16 samples collected from different parts of the plants such as leaf, bark or root of. Four types of solvents with different polarity were used producing 64 extracts (extract library). Disk diffusion method was used for antibacterial screening using five bacterial strains. The results showed that some extracts were active against gram negative bacteria and some others were active against bacterial positive bacteria. Some extracts others did not show any antibacterial activity against tested bacteria. Overall results indicated that mangrove extracts were potential antibacterial agents.
Lichens of Java Island showing anti-bacterial and anti-cancer activities

Ari Satia Nugraha1,2*, Dwi Koko Pratoko1, Yuvita Dian Damayanti1, Nadya Dini Lestari1, Tinton Agung Laksono1, Hardian Susilo Addy2, Ludmilla F. Untari3

1Drug Discovery Research Group, Faculty of Pharmacy, University of Jember, Jember, Indonesia 68121; 2Center for Development of Advance Science and Technology, University of Jember, Jember, Indonesia 68121; 3Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia 55281

*Presenting author
Email: asn559@uowmail.edu.au, Phone: +62 81391000208

ABSTRACT

Lichen is a unique composite organism arises from algae and fungi symbiotic relationships. Lichen growth from sea level to mountain and from polar to tropical region which 18,500 recorded species worldwide and new record is counting. Indonesia is the largest archipelagic and the second largest biodiversities in the world including the lichens. The research focused on anti-microbial and anti-cancer screenings on the lichens of Java collected from several Districts in Java including Jember, Bondowoso, Pasuruan and Malang. Seventeen lichens were collected and identified, and their crude methanol extracts were tested against gram-negative bacteria and several cancer cell lines (MCF7, Widr, Hela). Methanol extracts of Parmelia cetrata Ach. and Parmelia dilatata Vain, possessed a moderate activity against Pseudomonas aureginosa. Hela cell line was sensitive to crude methanol extracts of Physcia cf. millegrana Degel., Parmelia caroliniana Nyl. With IC50 of 137 and 328 ug/mL, respectively. Methanol extracts of Cladonia scabriscabruscula (Duby) Leight was cytoxic against MCF7, Widr and Hela cell lines. Overall, further research is required to reveal active constituents of the lichens.

Keywords: Lichen, Java, anti-microbes, anti-cancer
Alstonville (*Tibouchinalepidota*), a Purple Flower, as Antibacterial Agent

Rudi Hendra\textsuperscript{1,2,*} and Paul Keller\textsuperscript{2}

\textsuperscript{1}Department of Chemistry, Faculty of Mathematic and Natural Sciences, University of Riau, Indonesia;  
\textsuperscript{2}School of Chemistry, University of Wollongong, NSW, Australia

\*Presenting author  
Email: rudi.hendra@lecturer.unri.ac.id, Phone: +6281365340190

**ABSTRACT**

**Background:** Australia has a hugely variable climate with arid, tropical, desert, alpine, and extreme conditions extending across the country. Australian plants have therefore developed a wide variety of survival methods specific to their environmental challenges, and as such there are many unique morphologies and phenotypes present. This includes not only endemic plants, but also the large range of introduced species, some of which are even cultivated. As well as some Australian plants (endemic and introduced) being used as medicine, some also possess unique and colourful flowers, with examples such as Alstonville (*T. lepidota*). *T. lepidota* is an introduced plant in Australia, where it is cultivated. This species is used as a medicinal plant in South America for wound healing, antiseptic, and antimicrobial purposes. From the previous work, seven secondary metabolites were isolated from the flower of this species, however biological activities contained within the flowers from this species are limited.

**Objective:** In this study, the investigation of antibacterial activities from several isolated compounds from previous work within the flower of this species was conducted.

**Methods:** From previous works, several flavonoids and two phenolic acids were isolated from the methanol extract of the flower. These compounds and methanol extract from the flower were applied for their antimicrobial activities by using Hit confirmation antimicrobial assay against pathogenic bacteria (*Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853)).

**Results:** Methanol extracts and all the compounds showed various results to inhibit pathogenic bacteria growth with single concentration at 32 µg/mL. The extract as well as including quercetin and malvidin 3-(coumarylglucoside)-5-(acetylxyloside) showed high antibacterial activities with percentage of inhibition value 57.90%, 56.26%, and 60.77%, respectively against *S. aureus*. Furthermore, the extract and these compounds show high antibacterial activities against *A. baumannii* with percent inhibition values of 55.12%, 60.59%, and 51.41%, respectively.

**Conclusion:** It can be concluded that not only the species possess bright colourful flowers, but also show antimicrobial activity. Further investigations are needed to obtain mode of action from these compounds to inhibit the growth of bacteria.

**Keywords:** *Tibouchinalepidota*, flavonoids, antibacterial
DAY 1-PARAREL ROOM 2
(R.300)-(OP 10-OP 18)
Isolation of a Flavonol and Two Phenolic Compounds from the Leaves of *Macarangahosei* King ex Hook.f.

Mohamad Hamizan Mohd Isa¹,²*, Norizan Ahmat¹,², and Aisyah Salihah Kamarozaman¹,²,³

¹Faculty of Applied Science, UiTM Shah Alam, 40450 Shah Alam, Selangor D.E. Malaysia; ²Atta-ur-Rahman Institute for Natural Product Discovery, UniversitiTeknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor D.E. Malaysia; ³Pusat Asasi UiTMDengkil 43800 Dengkil, Selangor D.E. Malaysia

*Presenting author
Email: hamizanisa93@gmail.com, Phone: +60127193071

ABSTRACT

**Background:** The genus of *Macaranga* from Euphorbiaceae family encompasses of approximately 308 species that are endemic to the tropics of Asia, Australia, Africa, and Pacific contingents. Some *Macaranga* species have emerged as a good source of ethnomedicine by aboriginal folks. Traditional practitioners use fresh or dried leaves of certain *Macaranga* species as anti-inflammatory medicine which are capable of treating cuts, sores, swellings, boils and bruises. Nevertheless, most flavonoids from *Macaranga* species are generally isoprenylated, farnesylated, geranylated, or geranylgeranylated.

**Objective:** To isolate and characterize the chemical constituents from the methanolic extract of the leaves of *Macarangahosei* by using several chromatographic techniques and subjected them to various spectroscopic methods.

**Methods:** The leaves of *M. hosei* were collected from reserved forest Lenggong, Perak, Malaysia, and the dried and powdered leaves were macerated in methanol at room temperature. Then, the crude methanolic extract was fractionated by using vacuum liquid chromatography (VLC) to give seven (7) major fractions (MH1-MH7). Fraction MHL3 was further purified by series of column chromatography (CC) and a flavonol, namely Ombuin (1) was purified by using recrystallization method. Vanillic acid (2) and 3’4’-dihydroxypropiophenone (3) were isolated by using semi-preparative HPLC. The chemical structure of these isolated compounds was characterized based on their 1D and 2D NMR, UV, and MS spectral data.

**Results:** Phytochemical study on the methanolic extract of the leaves of *M. hosei* has yielded three compounds known as Ombuin (1), Vanillic acid (2), and 3’4’-dihydroxypropiophenone (3).

**Conclusion:** A flavonol and two phenolic compounds were isolated from the leaves of *M. hosei*.

**Keywords:** *Macarangahosei*, flavonoid, phenolic compound, Euphorbiaceae, Ombuin, Vanillic acid, and 3’4’-dihydroxypropiophenone.
Identification and Determination of Total Flavonoid Content of Extract Pasak Bumi (Eurycoma longifolia Jack) Root by FTIR and Multivariat Calibration

Liling Triyasmono*, Khoerul Anwar, M Ikhwan Rizky, Ana Ulfa, Isni Munisa

Department of Pharmacy of Lambung Mangkurat University

ABSTRACT

The combination of FTIR and multivariate calibration is an alternative method on determination of identification and total flavonoid content of extract pasak bumi (Eurycoma longifolia Jack.) roots. The aim of this study is to apply FTIR method and multivariat calibration for autentification and determination of total flavonoid content of extract E. longifolia roots. The analysis was done by Principal Component Analysis (PCA) chemometrics which aimed to grouping IR spectrum based on growing area and Partial Least Square Regression (PLSR) which was useful to determine prediction model of total flavonoid content of extract E. longifolia roots. Determination of prediction model was done by using absorbance of FTIR measurement result as predictor variable (x) and total flavonoid content from measurement by standard method as response variable (y). The best grouping and prediction model is shown by the 9th model (preprocessed IR spectrum in the range of wave numbers (1800-1540 cm⁻¹)). The result of analysis showed total variation is 99% (PC1 = 94%, PC2 = 5%) and prediction model with equation \( y = 0.995x + 0.002 \) (\( R^2 \) calibration = 0.995; \( R^2 \) validation = 0.967; RMSEC = 0.008; RMSECV = 0.021). The prediction model was tested to the sample and obtained the total flavonoid content of extract E. longifolia roots from Mandiangin, Condong and Sabuai is 0.224%; 0.437% and 0.465% with \( R^2 \) and RMSEP of 0.995 and 0.008. Based on this, the combination of FTIR and chemometrics can be used to predict the total flavonoid content of unknown extract E. longifolia roots.

Keywords: Eurycoma longifolia Jack., Flavonoid, FTIR, PCA, PLSR.
Two flavones and two phenolic compounds from *Asystasiagangetica* (L) T. Anderson var. micrantha (Acanthaceae)

Isna Athirah Othman\(^1,2\)*, Norizan Ahmat\(^1,2\)

\(^1\)Faculty of Applied Science, UiTM Shah Alam, 40450 Shah Alam, Selangor D.E. Malaysia; \(^2\)Atta-ur-Rahman Institute for Natural Product Discovery, UniversitiTeknologi MARA (UiTM), PuncakAlam Campus, 42300 Bandar PuncakAlam, Selangor D.E. Malaysia

*Presenting author
Email: isnaathirah@gmail.com, Phone: +60145177918

ABSTRACT

**Background:** *Asystasiagangetica* (L) T. Anderson var. micrantha (Acanthaceae) or commonly known as Chinese violet or rumput Israel is a straggling herb usually found among short grasses and along pathways. This plant used traditionally to treat diabetes mellitus, ear disease, gonorrhea, and its anthelmintic activity helps to treat swelling and rheumatism.

**Objective:** The present study was designed to isolate and elucidate bioactive compounds from *A. gangetica*.

**Methods:** Methanolic extract of the *A. gangetica* leaves was fractionated by using PLC. Selected fractions were subjected to preparative HPLC and recycling HPLC for further purification. All of the compounds were monitored by using UHPLC. The structures of isolated compounds were characterized by using spectroscopic method including NMR, IR, UV and mass spectral data.

**Results:** Four phytochemical constituents namely chrysoeriol, 5,7,4'-trihydroxy-3'-methoxyflavone-4'−O-\(\beta\)-D−glucoside, (E)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid and methyl (E)-3-(3,4-dihydroxyphenyl)acrylate have been isolated from *A. gangetica*. All of the compounds were identified for the first time from genus *Asystasia*.

**Conclusion:** Two flavones and two phenolic compounds were isolated from *A. gangetica*.

**Keywords:** *Asystasiagangetica*; flavone; phenolic compound; HPLC; NMR
ABSTRACT

Background: Radical compounds in the body are very reactive to cells that can cause various diseases such as cancer, arteriosclerosis and aging. Compounds that are able to counteract free radicals are antioxidants. Antioxidants can be obtained from fruits and plants.

Objective: This study aims to determine the antioxidant capacity of fruit methanol extract and Tiin leaf.

Methods: The extraction was performed by maceration method with methanol solvent at room temperature for 3 days. The extraction results in the phytochemical test and functional group analysis to determine the compounds contained in fruit extract and Tiin leaf. Further testing of antioxidant activity with DPPH method. Phytochemical test and functional group analysis to see the content of compounds contained in extracts such as flavonoids, Triterpenoids and Sterols, Alkaloids and Saponins.

Results: Tiin leaf extract has antioxidant activity with IC\textsubscript{50} value of 48.9 μg/ml while Tiin fruit extract has antioxidant activity of 102.7 μg/ml. The IC\textsubscript{50} value <50 μg/ml indicates that the extract has antioxidant potential.

Conclusion: Tiin leaf extract is more potent to antioxidant activity compared with Tiin fruit.

Keywords: antioxidant, phytochemical, extractTiin (Ficuscarica).
Benzopyrones and Fatty Acids from Ethyl Acetate Layer of *Spilanthes acmella* Murr

Retno Widyowati*, Wiwied Ekasari, and Neny Purwitasari

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya 60285

*Presenting author
Email: rr-retno-w@ff.unair.ac.id, Phone: +6281615886978

ABSTRACT

**Background:** The main constituents from the whole aerial parts, flower heads and roots of *Spilanthes acmella* Murr. (Compositae) yield “spilanthol” and “acmellonate,” they are sometimes used to reduce the pain associated with toothaches, induce saliva secretion and is a powerful insecticide and local anesthetic. It also has an important source of highly valuable bioactive compounds such as fatty acids, phenolics, coumarin (scopoletin), triterpenoids and flavonoids.

**Objective:** This research has purpose to find the chemical constituents containin the ethyl acetate layer of the 70% ethanol extract of *Spilantel acmella*.

**Methods:** The structures of these compounds were isolated by various chromatographic techniques such as silica gel, ODS, HPLC and determined as follows by spectrometric analysis (UV, IR, HR-ESI-MS, 1D and 2D NMR) and based on chemical evidences.

**Results:** The investigation of the ethyl acetate layer of this plant has demonstrated the present of a benzene propanoic (1), 9,11-octadecadienoic methyl ester acid (2), tridecanoic methyl ester acid (3), and pentadecanoicacid (4).

**Conclusion:** The ethyl acetate layer of *Spilanthes acmella* contains benzopyrones and fatty acids.

**Keywords:** *Spilanthes acmella*, benzopyrones, fatty acids.
OP15

Antibacterial Activity of Methanol Extracts and Compounds of Wualae (Etlingera elatior) Fruits from Southeast Sulawesi-Indonesia

Sahidin I1, Syefira Salsabila1, Wahyuni W1, M. Hajrul Malaka1, Imran2 and Marianti A. Manggau3

1Faculty of Pharmacy, Universitas Halu Oleo Kendari 93232 Southeast Sulawesi, Indonesia;
2Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Halu Oleo Kendari 93232 Southeast Sulawesi, Indonesia;
3Pharmacology, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, South Sulawesi, Indonesia

*Presenting author
Email: sahidin02@yahoo.com

ABSTRACT

Wualae (Tolakinese) or Etlingera elatior grows bulky in Southeast Sulawesi. The fruit of this plant is widely used as cooking spices and traditional medicine. Scientific studies of the fruit and its properties against certain diseases are still very limited. The aim of the article shares the chemical content of E. elatior fruits and its activity against various selected pathogenic bacteria. Isolation was performed by chromatographic techniques, including Thin Layer Chromatography (TLC), Vacuum Liquid Chromatography (VLC), and Radial Chromatography (RC). Structure of the isolated compounds was elucidated by using spectroscopic techniques, i.e. IR and NMR-1D spectroscopy (1H and 13C-NMR) and comparing with similar data from the literature. The activity of the methanol extracts and the isolated compounds were evaluated against bacteria using the diffusion agar method. The tested bacteria included Escherichia coli ATCC 35218, Pseudomonas aeruginosa, Staphylococcus aureus ATCC 25923, Bacillus subtilis, Streptococcus mutans ATCC 25175 and Salmonella enteric. The result showed that two compounds have been isolated from E. elatior fruit, namely vanilic acid and p-hydroxybenzoic acid. The compounds and crude extracts were most active against S. mutans. The data is a reference where the methanol extracts of E. elatior fruits can be developed into a mouthwash or toothpaste.

Keywords: Etlingera elatior, fruits, vanilic acid, p-hydroxybenzoic acid and antibacteria
Acetylcholinesterase Inhibitory and Radical Scavenging Activities of Oligostilbenoids from *Shorea leprosula* Miq.

Aisyah Salihah Kamarozaman¹,²,³*, Norizan Ahmat¹,³, Fatin Nur Syahirah Mohamad Sharif¹, Nurul Atikah Azmi¹, Muhammad Sulaiman Mohd Johari¹, Zetty Zulikha Hafiz¹,³, Mohd Ilham Adenan¹,³

¹Faculty of Applied Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; ²Centre of Foundation Studies, Universiti Teknologi MARA, Selangor Branch, Dengkil Campus, 43800 Dengkil, Selangor, Malaysia; ³Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

*Presenting author
Email: aisyahsalihah@yahoo.co.uk, Phone: +60192109614

ABSTRACT

Background: Dipterocarpaceae comprises species of high economical values in timber, resins, oil, camphor as well as turpentine. *Shorea* which belongs to this family consists of 196 species and mainly found in Southeast Asia. Besides being of importance in timber production, this genus is known for their potential in pharmaceutical industries due to its significant bioactivities. Thus, in this research, *Shorea leprosula* or Light Red Meranti was chosen to be phytochemically studied.

Objectives: To isolate and elucidate the oligostilbenoids from the stem bark of *S. leprosula*; To examine the acetylcholinesterase inhibitory (AChEI) and DPPH radical scavenging activities of the oligostilbenoids isolated.

Methods: The stem bark of *S. leprosula* was collected from Hutan Simpan Gombak, Selangor and the voucher specimen (SK1634/09) was deposited at Herbarium of Universiti Putra Malaysia. The powder of the stem bark (2.76 kg) of *S. leprosula* was macerated in acetone for 24 hours at room temperature and repeated five times. The concentrated extract was mixed with diethyl ether to remove the tannin. The less tannin fraction of the acetone crude extract (82.3 g) was semi-purified by vacuum liquid chromatography (hexane:ethyl acetate) to give seven fractions (SL1-7). Fraction SL4 (423.5 mg) was further purified by radial chromatography (chloroform:methanol) to afford a pure compound (1) (31.6 mg). SL6 (150.0 mg) and SL7 (270.7 mg) were subjected to the same chromatographic technique and solvent system to yield two pure compounds (2) (10.9 mg) and (3) (8.6 mg) respectively. The isolates were tested on AChEI and DPPH radical scavenging activities.

Results: Based on the UV, IR, NMR and comparison with the literature data, the isolated compounds were characterized as hopeaphenol (1), isohopeaphenol (2) and laevifonol (3). Compound (1) displayed pronounced activity on AChEI and DPPH radical scavenging with the IC₅₀ 10 μM and 7.02 μM respectively. Meanwhile, compound (3) only exhibited moderate activity of AChEI with the IC₅₀ >20 μM.

Conclusion: The phytochemical study on the stem bark of *S. leprosula* has successfully yielded three oligostilbenoids namely hopeaphenol (1), isohopeaphenol (2) and laevifonol (3). Among the isolates tested, hopeaphenol (1) exhibited good activity in both AChEI and DPPH radical scavenging activities.

Keywords: *Shorea leprosula*, oligostilbenoids, radical scavenging, acetylcholinesterase inhibitory.
Estrogenic Activity Potency of Four Isolated Compounds from Ethyl Acetate Extract of Sasaladaan (Peperomia pellucida [L.] Kunth)

I Gusti Agung Ayu Kartika1*, Kyu Hyuck Chung2, Jong Hwan Kwak3, Catur Riani4, Muhamad Insanu5, I Ketut Adnya1

1Pharmacology and Clinical Pharmacy Department, School of Pharmacy, Institut Teknologi Bandung, Ganesha 10 Bandung, 40132, Indonesia; 2Prevent Pharm Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon-Si, Gyeonggi-Do, South Korea; 3Phytochemistry Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon-Si, Gyeonggi-Do South Korea; 4Pharmaceutics Department, School of Pharmacy, Institut Teknologi Bandung, Ganesha 10 Bandung, 40132, Indonesia; 5Pharmaceutical Biology Department, School of Pharmacy, Institut Teknologi Bandung, Ganesha 10 Bandung, 40132, Indonesia

*Presenting author
E-mail: kartikaayu269@gmail.com, Phone: +6282235478779/+6281937411685

ABSTRACT

Background: Several studies have been conducted to prove the potential of sasaladaan (Peperomia pellucida [L.] Kunth) extracts as an anti-osteoporosis and anticancer but there is no report about the level of its estrogenic activity which has an important role in those two activities.

Objective: Therefore, this study aims to measure the estrogenic potency of ethyl acetate extract and four isolated compounds from sasaladaan.

Methods: This study was started with isolation several compounds from ethyl acetate extract. Then in vitro viability test, estrogenic and antiestrogenic activity test using E-SCREEN assay and ligand-receptor binding assessment using docking assay were conducted.

Results: In this experiment pellucidin A, dillapiol, one of dillapiole derivate, and apiole were successfully isolated. Viability test on MCF-7/BUS gave 80% or more with doses lower than 10 μg/mL. Ethyl acetate extract, its fractions, and the isolated compounds were proved to have the estrogenic activity which can classify as partial agonist to full agonist, but all of them except dillapiol have antiestrogenic activity too. Apiole has the highest estrogenic activity, even higher than its extract. Based on the estrogenic test using tamoxifen and docking test to the estrogen receptor, the estrogenic activity of the isolated compounds was proven to be mediated by classical ligand-receptor binding interactions even though their binding energy were quite low.

Conclusion: The various level of estrogenic and antiestrogenic activity of ethyl acetate extract and its fractions most likely came from the interaction between single chemical compounds and that activity maybe mediated by not only one mechanism.

Keywords: docking, estrogenic, isolation, peperomia pellucida
Anti-inflammatory Activity of Catechins Isolate of Uncaria Gambir ROXB in Albino Ratsthat Induced with Carrageenan

Muhammad Yanis Musdja*, Nelly Suryani, Pipit Pitriyah

Department of Pharmacy, Faculty of Medicine and Health Sciences, State Islamic University, Syarif Hidayatullah, Jakarta, 15412.

*Presenting author
Email: yanis.musdja@uinjkt.ac.id, myanis88@gmail.com, Phone: +6281281681197

ABSTRACT

Background: Uncaria gambir Roxb (gambir) is often used by some communities in Indonesia and other countries in Southeast Asia and South Asia to treat inflammation and other treatments. Catechins as the main content of the gambir are allegedly powerful that have anti-inflammatory properties. The objective of this study was to determine the anti-inflammatory activity of catechins isolates of gambir in albino rats that induced with carrageenan.

Methods: Catechins of gambir were isolated by using partition method with ethyl acetate solvent. A total of 25 male albino rats were divided into 5 groups. Tests of anti-inflammatory effects was done based on Winder's modified paw edema method. Rats were made to suffer inflammation by the injected with carrageenan 0,1 ml of 1 % (b/v). The effect of edema on rat's foot was done 1 hour after administration of test preparation for 6 hours by using plethysmometer. A total of 3 groups were given catechin isolates per oral with a dose of 1 mg/kg BW, 10 mg/kg BW and 100 mg/kg BW respectively. Test preparations were suspended in Na-CMC 0,5 %, As positive control was used Na-diklofenak and as negative control was used Na-CMC 0,5 %.

Results: Based on the results of statistical tests with ANOVA method was obtained a dose of 100 mg / kgBB gives the greatest anti-inflammatory effect, ie 59.19%. while for dose of 1 mg / kg, BW and 10 mg / kg BW also was happened a difference that significant compared with negative control (p≤0,05). The dose of 10 mg / kgBB have same effect with dose 1% Na-diclofenac.

Keywords: Uncaria gambir, Catechins isolate, anti-inflammatory, albino rats
DAY 1-PARAREL ROOM 3 (R.301)-(OP 19-OP 27)
Anti-inflammatory properties of *Scurrula ferruginea* parasitizing on three different host plants evidenced via metabolomic approach

Intan Safinar Ismail, Xia Hong, Sharida Fakurazi and Nurfiza Mat Isa

*Universiti Putra Malaysia, Selangor, Malaysia*

*Presenting author*
Email: safinar@upm.edu.my, Phone: +603-8946-7492

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**ABSTRACT**

The strategies for the identification of bioactive constituents from a plant crude extract has been changing from time to time. Despite the fact that the reductionist - bioassay guided approach is the most popular technique currently in practice, at times this method failed to deliver a holistic information about the therapeutic potential of various chemical constituents of a crude extract. It is specifically because of its inability to identify the effect of synergism or antagonism, which is considered as the most important attribute of an herbal preparation. Metabolomics being a global and comprehensive tool could overcome this disadvantage. In a study, the exogenic effect of three different hosts’ plants, *Vitex negundo* L., *Micromelum minutum* (G. Forst.) Wight & Arn. and *Tecoma stans* (L.) Juss ex HBK on *Scurrula ferruginea* along with the mechanism of *S. ferruginea* in inducing the anti-inflammatory ability were elucidated. *S. ferruginea* stems parasitizing on *T. stans* and *V. negundo* which were freeze dried exhibited higher anti-inflammatory activity than that from *Micromelum minutum*. The mid-polar ethyl acetate fraction of *T. stans* displayed the highest NO inhibition. *S. ferruginea* exerted its anti-inflammatory properties in inhibiting iNOS and IL-1β gene and protein expression probably could be suggested through the blocking of NF-κB activation. Principal component analysis (PCA) indicated notable and clear discriminations among the different groups suggesting the contributed metabolites, which were responsible for the differentiation clustering. Furthermore, partial least square regression (PLS) model suggested that the anti-inflammatory bioactivity might be associated with the presence of primary and secondary metabolites including choline, isoleucine, catechin, leucine, and chlorogenic acid. This study has shown a remarkable medicinal evidence of some traditional plants via metabolomics approach.
ABSTRACT

The loss of vasculoprotective effects of estrogen is the cause of an increased incidence of hypertension in postmenopausal women. The red beans (Vigna angularis L) contain phytoestrogens. The objective of the study was to prove the effect of red beans as vasculoprotective in hypoestrogenic rats. The research design used Post Group Control Only Control. The samples of female Wistar rats aged 10-12 weeks, weight 125-150g, divided into normal group, ovariectomy and ovariectomy treated and given red bean ethanol extract dose 2.5mg / KgBB, 7.5mg / KgBB and 22.5mg / KgBB during 30 days by nasogastric sonde. Each group consists of 5 rats. At the end of the treatment is done sacrifice and organ harvesting. Circulating Endothelial Cells Examination using flowcytometry method, measurement of the area of collagen aorta tissue using histology preparation with Masson Tricom's staining and measurement of N0 rat tail rate using Nitrate / Nitrite Colorimetric Assay Kit. The area of collagen aorta is observed using Dot Slide microscope, 100x magnification for the area of collagen. The measurement of area of collagen aortic was calculated using image software J. Data analysis of CEC using Kruskal-Wallis and and continued with Mann Whitney, p <0.05. Data analysis of the area of collagen aorta and N0 rat tail using One Way Anova method and continued with BNT test, p≤ 0.05. Supplementation of red bean extract dose 7.5mg / KgBB, and 22.5mg / KgBB increased NO content by 30% (p <0.05), decreased amount of Circulating Endothelial Cells (CECs) by 52% and 47%, and decreased the area of collagen aorta of 18% and 33% (p <0.05) on hypoestrogen female Wistar rats. The conclusion was obtained that the extract of red bean ethanol (Vigna angularis L) had vasculoprotective effect on hypoestrogen female Wistar rats.

Keywords: Estrogen, Vigna angularis, Circulating Endothelial cells, Nitric Oxide, Collagen of aorta, ovariectomy rats
In Vitro Study of *Garciniacelebica* L.Barks against Hepatitic C Virus and Hepatocellular Carcinoma

Dadan Ramadhan Apriyanto¹*, Sri Hartatic², Beti Ernawati Dewi³, Chie Aoki-Utsubo⁴, Hak Hotta⁵

¹Division of Microbiology, Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia;
²Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan Puspiptek, Serpong 15310, Indonesia;
³Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia;
⁴Department of International Health, Kobe University Graduate School of Health Sciences, 7-10-2, Tomogaoka, Suma-kō, Kobe 654-0142, Japan;
⁵Department of Oral Vaccine and Drug Development, Kobe University Graduate School of Health Sciences, 1-5-6 Minatojima-minamimachi, Chou-ku, Kobe 650-0047, Japan

*Presenting author
Email: daniramadhana@gmail.com, Phone: +6289687304227

ABSTRACT

**Background:** Chronic infection Hepatitis C Virus (HCV) can develop into hepatocellular carcinoma (HCC) which can lead to liver dysfunction and death. HCV chronic infections and HCC are the world's problems that can not be overcome simultaneously. Recently, the standard therapy is also associated with significant adverse effects and high costs limit the broad use of the therapy. Development of antiviral drugs and anticancer for the treatment of Hepatitis C with HCC obtained from medicinal plants is an alternative or additional treatment in the therapy.

**Objective:** The acetone of *Garcinia celebica* L. from barks in Indonesia were examined in terms of their inhibitory effect on HCV replication and hepatocellular carcinoma.

**Methods:** The extractions used maceration method were started from n-hexane solvent and the residue continues used acetone solvent. The extracts were then examined with several doses 20, 10, 5, 2.5, and 1.25 μg/mL for antiviral activity against JFH1 strain genotype 2a and anticancer cytotoxicity against Huh7it-1 cell line.

**Results:** The extracts exhibited anti-HCV activity on 1.25 μg/mL without toxicity to Huh7it-1 cells. The extracts exhibited Anticancer activity on 20, 10, 5, and 2.5 μg/mL with significant toxicity to Huh7it-1 cells.

**Conclusion:** These results suggest that *Garcinia celebica* may be useful as an add-on therapy candidate for treating HCV infections and HCC.

**Keywords:** hepatitic C virus, antivirus, hepatocellular carcinoma, *Garcinia celebica*
ABSTRACT

In this research, study of 8-hydroxy-2′-deoxyguanosine (8-OHdG) caused by exposure of Chromium (III) and Benzo[a]pyrene was conducted. This study was done by reacting 2′-deoxyguanosine as DNA base with xenobiotic like Benzo[a]pyrene with variation of pH (7.4 and 8.4), incubation time (7 and 12 hours), and incubation temperature (37°C and 60°C). On this mixture, another observation was conducted with addition of Chromium (III) and H₂O₂ as the Fenton-like reaction reagent. 8-OHdG DNA Adduct was then analyzed with High Performance Liquid Chromatography (HPLC) reversed phase with UV detector on 245 nm wavelength. The mixture of pH 6.7 Phospate Buffer 10mM and LC-grade methanol with ratio of 85:15 and 1 mL/minute flow rate were used in the measurement of 8-OHdG. On every mixture in all pH, time, and temperature variation, 8-OHdG was detected with the concentration below the Limit of Quantification, thus the concentration cannot be quantified. Addition of the Fenton-like reaction reagent also impacted on higher 8-OHdG concentration in result. Longer incubation time and higher incubation temperature were proved to generate more 8-OHdG, meanwhile the variation of pH did not significantly affect the concentration of generated 8-OHdG in the mixture.

Keywords: Benzo[a]pyrene, DNA Adduct, 8-OHdG, Fenton-like Reaction
Induction of apoptosis in human colorectal cancer (HT-29) cell line by Manilkara zapota leaf water extract via modulation of caspase-dependent pathway and antioxidant activity

Norhaizan ME1,2,3*, Tan BL1, SitiNursalwah CO1, and NurulHusna Shafie1

1Department of Nutrition and Dietetics, 2Research Centre of Excellent, Nutrition and Non-Communicable Diseases (NNCD), Faculty of Medicine and Health Sciences, 3Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

*Presenting author
Email: nhaizan@upm.edu.my, Phone: +603 89472427

ABSTRACT

Background: Manilkara zapota also called as ciku in Malaysia and sawo in Indonesia has been used as traditional folk medicine to treat various diseases.

Objectives: This study was aimed at investigating the antioxidant and cytotoxicity of Manilkara zapota. The underlying mechanisms of Manilkara zapota extracts induced cytotoxicity in human colorectal cancer (HT-29) cells was also evaluated.

Methods: The total phenolic and flavonoids contents and β-carotene bleaching test and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) were evaluated in different parts (flesh, peel, leaf, and seed) of Manilkara zapota water and 70% ethanol extracts. Phenolic compounds were determined using Ultra Performance Liquid Chromatography (UPLC). The cytotoxicity of Manilkara zapota leaf extracts was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. The morphological changes in HT-29 cells treated with the extract was observed under inverted light and fluorescence microscope. The apoptotic cells were measured by Annexin V-propidium iodide staining whereas caspases were determined by colorimetric assay.

Results: Overall analyses revealed that Manilkara zapota leaf water extract showed the highest total phenolic and flavonoid contents and cytotoxic activities than 70% ethanol extract. Incubation of Manilkara zapota leaf water extract significantly increased the total apoptotic of HT-29 cells (p < 0.05). Incubation HT-29 cells with Manilkara zapota leaf water extract for 72 h also induces apoptosis by the significant elevation (p<0.05) of caspase-3 and -8 activities compared to the control. The presence of flavonoids and saponins as well as other polyphenolic constituents are believed to contribute to this effect.

Conclusions: Our results provide evidence that Manilkara zapota leaf water extract offer great potential against colorectal cancer via induction of apoptosis in HT-29 cell line by modulation of caspase-dependent pathways and antioxidant levels.

Keywords: Manilkara zapota, colon cancer (HT-29), antioxidants, apoptosis, caspase.
The Antioxidant Activity of Ethanolic Extract and Fractions of *Hibiscus surattensis* L Leaves

Yuliet¹*, Elin Yulinah Sukandar¹, and I Ketut Adnyana¹

¹Department of Department of Pharmacology and Clinical Pharmacy, Bandung Institute of Technology, Bandung 40116, Indonesia

*Presenting author
Email: yuliet_susanto@yahoo.com, Phone: +6282195213868

ABSTRACT

**Background:** *Hibiscus surattensis* L has been used for its antimalarial, wound healing, antihypertensive, venereal disease and urethritis effects in folk medicine. However, little is known about how it works to produce these therapeutic actions. Since these effects may be correlated with the presence of antioxidant compounds, ethanolic extracts of *H. surattensis* L leaves were evaluated for their antioxidant activity.

**Objective:** The present study was designed to confirm the leaves of *H. surattensis* L as an antioxidant agent.

**Methods:** Ethanolic extract (EE) and its n-hexane (NHF), ethyl acetate (EAF) and water (AQF) fractions were screened for in vitro antioxidant activity, total phenolic, and total flavonoid content. Evaluate antioxidant activities using in vitro methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH), CUPRAC (cupric ions reducing assay), and FRAP (ferric reducing antioxidant power). The total phenols and flavonoids have been determined for all studied extracts/fractions by spectrophotometric methods.

**Results:** In DPPH radical scavenging assay, EAF exhibited highest scavenging activity (IC⁵⁰ = 44.10 μg/mL) as compared to EE and other fractions. In this assay, IC⁵⁰ of reference standard quercetin was 6.48 μg/mL and ascorbic acid was 4.56 μg/mL. The CUPRAC and FRAP assay of the samples was in the order as EAF > EE and NHF > AQF. In the assay of total phenol and total flavonoids content, the EAF had highest phenolic and flavonoids content (329.23 mg GAE/g and 684.67 mg QE/g).

**Conclusion:** According to the present study, EAF can be used as the natural antioxidants source to prevent diseases associated with free radical.

**Keywords:** *Hibiscus surattensis* L, antioxidant activity, extract, fraction.
Antiviral Effect of Sub Fraction of *A. heterophyllus* Leaves Extract to Hepatitis C Virus (JFH1) and its Mechanism of Action

Adita Ayu Permanasari 1*, I Putu Abhiseka Pranajaya 2, Lydia Tumewu 1, Myrna Adianti 1,3, Tutik Sri Wahyuni 1,2, Sri Puji Astuti Wahyuningsih 4, Aty Widyawaruyanti 1,2, Achmad Fuad Hafid 1,2

1 Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia; 2 Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia; 3 Department of Health, Study Program Traditional Medicine, Vocational Faculty, Universitas Airlangga, Surabaya, Indonesia; 4 Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia

*Presenting author

Email: adita_united88@yahoo.com, Phone: +6282331248876

ABSTRACT

Background: Hepatitis C virus (HCV) infection is one of the causes progressive liver damage, which often lead to liver cirrhosis and hepatocellular carcinoma. Currently therapy for HCV infection is very expensive and possibility has less effectiveness caused by resistance and mutations of HCV. From the results of previous studies, the dichloromethane extract from *A. heterophyllus* leaf was reported to have the ability to inhibit HCV with Inhibition Concentration 50% (IC 50) value of 1.5 µg/mL and Cytotoxicity Concentration 50% (CC 50) value of >200 µg/mL.

Objective: To investigate the inhibition mechanism of *A. heterophyllus* active fraction as anti HCV from *A. heterophyllus* dichloromethane extract.

Methods: Dichloromethane extract of *A. heterophyllus* leave were fractionated using VLC and HPLC. The fractions and subfractions was analyzed for antiHCV activity and their toxicity in hepatocyte cells Huh7it and HCV JFH1 (genotype 2a). The mechanism analysis of fractions and subfractions was performed by time addition experiment, virucidal assay, expression of host receptor assay, HCV absorption assay, protein expression assay, and total relative RNA assay.

Results: 4 fractions (FR1DCM-FR4DCM) and 7 sub fractions (FR3T1-FR3T7) from *A. heterophyllus* dichlormethane extract were obtained. Fraction 3 (FR3DCM) was known as the most active fraction with IC 50 3.79 ± 2.35 µg/mL and CC 50 > 100 µg/mL (SI value > 26.39). One of active subfractions (FR3T3) showed activity to inhibit HCV with IC 50 4.69 ± 0.95 µg/mL and CC 50 >100 µg/mL (SI value >21.32). A time-of-addition studies showed that FR3T3 subfraction inhibited HCV in viral entry process of 61.68 ± 0.10% and post entry step of 83.86 ± 2.58%. The entry step inhibition occurredly high at concentrations of >20 µg/mL through direct virucidal effect and affecting host receptor. On the other side, the post entry step inhibition of FR3T3 was indicated by decreasing of NS3 and NS5A protein production as well as the relative amount of HCV’s RNA.

Conclusion: The FR3T3 subfraction from *A. heterophyllus* dichloromethane extract is a good candidate to develop an antiviral agent to prevent HCV infection.

Keywords: Hepatitis C Virus (HCV), Artocarpus heterophyllus, antiviral.
Antioxidant activity of *Lygodium microphyllum* Aerial Parts

Hadi Kuncoro*

Laboratorium Penelitian dan Pengembangan Kefarmasian Farmaka Tropis, Faculty Of Pharmacy, Muawarman University, Samarinda 75117, Kalimantan Timur, Indonesia

*Presenting author
Email: hadikuncoro@farmasi.unmul.ac.id, Phone: +62541739491

ABSTRACT

**Background:** *Lygodium microphyllum* is an invasive plant known as “Old World Climbing Fern” or is considered a weed plant. Research on *Lygodium microphyllum* related pharmacological activity is limited.

**Objective:** The purpose of this study is to evaluated antioxidant activity from Methanol extract and Fractionation using *n*-hexane, Ethyl acetate and water solvent with different polarity from *Lygodium microphyllum* plant.

**Methods:** Aerial part of *L. microphyllum* was macerated with methanol solvent, the extract obtained in fractionation using solvents with different polarity levels and was tested in invitro free radical using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

**Results:** *IC$_{50}$* result was 49.328 ± 0.242 ppm for methanol extract, *n*-hexane extract was 32.041 ± 0.770 ppm, ethyl acetate extract 8.732 ± 0.204 ppm and water extract 93.330 ± 1.931 ppm.

**Conclusion:** The study reveals the results of extraction using ethyl acetate solvent showed the highest value antioxidant activity against DPPH.

**Keywords:** *L. microphyllum*, Antioxidant, DPPH, *IC$_{50}$*
**ABSTRACT**

**Background:** Cancer is one of the biggest causes of death in the world, the secondary metabolite of marine biota is a new hope to overcome cancer. Agelas, sp., Callyspongia aerizusa and Callyspongia, sp. are sponges that commonly found in Indonesia that has a cytotoxic effect.

**Objective:** This study aims to determine the effect of methanol sponge extract Agelas, sp., Callyspongia aerizusa and Callyspongia, sp. on some cancer cells.

**Methods:** This *in vitro* study was performed on several cancer cells; TE-8 (Esophagus), Hep-G2 (Liver), Mia PaCa2 (Pancreas) and non-cancerous epithelial throat cells (HET-1A). The anticancer effect test of Agelas, sp., Callyspongia aerizusa and Callyspongia sp. Sponge extract was measured by MTS proliferation assay.

**Results:** The most cytotoxic sponge extract was Callyspongia sp., with IC$_{50}$ value for 24 h in TE-8, Hep-G2, Mia PaCa2 and HET-1A are 9 ng/ml, 3.22 µg/ml, 7.29 µg/ml and not detected, respectively. Whereas IC$_{50}$ value for 48 h are 6.3 ng/ml, 16.92 µg/ml, 3.1 ng/ml and not detected, respectively.

**Conclusion:** The most potential for anticancer agent was Callyspongia sp., methanol extract.

**Keywords:** Agelas, sp., Callyspongia aerizusa, Callyspongia, sp., anticancer, MTS proliferation assay.
DAY 1-PARAREL ROOM 4
(R.305)-(OP 28-OP 36)
Analysis of Albumin Levels in Farmed Sidat Fish (*Anguilla marmorata*) and Natural Breed Toman Fish (*Channamicropeltes*)

HariSantoso*1*

*1Department of Biology, FMIPA Unisma

*Presenting Author
Email: harisantoso.m.biomed@gmail.com, Phone: 082331449560

ABSTRACT

Sidat fish (*Anguilla marmorata*) is one of consumed fish species with relatively high nutrition contents, vitamins, micronutrients and albumin. Similarly, albumin is found in toman fish (*Channamicropeltes*) which is under the same family as gabus fish (*Channastriata*). In this research, sidat fish (*Anguilla marmorata*) is obtained from fish farms in Lamongan while toman fish (*Channamicropeltes*) is naturally obtained from Banjarmasin, South Kalimantan. The investigation is aimed at analysing the albumin level in sidat fish (*Anguilla marmorata*) found from fish farms and toman fish (*Channamicropeltes*) found naturally is swamps. The samples used for sidat fish and toman fish had average weight of 150-200 and 200 grams respectively. Using unpaired T-test from SPSS 23, the data analyses showed the average albumin level in sidat fish and toman fish is $\bar{X} = 8.032$ and $\bar{X} = 7.881$ respectively. This means that there is a significantly high difference in the albumin level found between sidat fish (*Anguilla marmorata*) and toman fish (*Channamicropeltes*).

**Keywords:** Albumin, *Anguilla marmorata*, *Channamicropeltes*
Physico-chemical characteristics, nutritional composition and phytochemical profiles of nine Algerian date palm fruit varieties: NMR-based metabolomics approach

Nur Ashikin Abdul Hamid1*, Faridah Abas1,2, Intan Safinar Ismail1,3 and Chau Ling Tham4

1Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
2Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Malaysia.
3Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
4Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

*Presenting author
Email: shikinhamid89@yahoo.com, Phone: +60173548860

ABSTRACT

Background: Date palm fruit (Phoenix dactylifera) is an economically important crop and has cherished for its nutritional and health benefits.

Objective: Many date varieties are marketed at various price ranges. However, there has not been any scientific method established to verify the quality of Algerian dates other than by basing it on their external observable features. Limited reports are available concerning their phenotypic characteristics, chemical constituents and pharmacological potentials. This study aims to assess the quality variations of nine selected varieties based on the metabolite composition, biological activities, proximate analysis and physical characteristics.

Methods: Nine Algerian date palm varieties; Lahmira, Timjouhert, Adham Talmine, Deglet Talmine, Adam Boullah, Tinasser, Deglet, Deglet Nour and Takerbouch were examined using Proton Nuclear Magnetic Resonance (1H-NMR) based metabolomics approach. The nitric oxide (NO) scavenging abilities, NO inhibition via the cell-based approach and total phenolic content (TPC) were determined. The physical features including dimension, seed/weight ratios and differences in colour were evaluated.

Results: The Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) revealed a consistent grouping of Deglet, Takerbouch and Deglet Noor from the rest of the six selected varieties. Among the metabolites that contributed towards the observed segregation were serine and glycine. Deglet dates demonstrated promising values for both NO inhibitory/scavenging activities.

Conclusion: The findings provide improved insight regarding the differences between Algerian date varieties. With regard to metabolite composition, NO-scavenging activities and proximate analysis, Deglet demonstrated a better quality hence may be suggested to be the candidate for the development of functional food.

Keywords: Phoenix dactylifera, Algerian dates, 1H-NMR metabolomics, date palm fruit varieties
Infra Red Spectroscopy and Multivariate Calibration for The Rapid Quantification of Free Fatty Acid Content in Pangasius Hypophthalmus Oil

Andina, L. 1*, Rohman, A. 2,3, Saputri, R. 1, Lukitaningsih, E. 2, Putri, A.N. 1

1Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari, Banjarbaru, 70714 Indonesia
2Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281 Indonesia
3Research Center of Halal Products, Gadjah Mada University, Yogyakarta, 55281, Indonesia

*Corresponding author: Lisaandina@stikesborneolestari.ac.id/ Lisa_imeru@yahoo.com

ABSTRACT

Free fatty acids (FFA) content in Pangasius hypophthalmus (catfish) oil was determined by attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy. Catfish oil derived from catfish’s meat (CMO) and catfish’s liver and fat (CLFO) were subjected to heat treatments. Determination of catfish oil’s fatty acid content was performed by Gas Chromatography-Flame Ionization Detector (GC-FID). Oleic acid was found to be the main fatty acid component in catfish oil, respectively. FTIR spectra of catfish oil have 3 main peaks, which shows stretching vibration of the C-H bonds due to the presence of cis-form of fatty acid, symmetric and asymmetric vibrations of the C-H2 and C-H3 aliphatic group and vibrations of the carbonyl (C=O) esters derived from the oil triglycerides. Partial least square regression (PLS) and principal component regression (PCR) calibration were developed to predict FFA content of Pangasius hypophthalmus oil. Based on the results, PCR model shows a better performance than PLS model. PCR at 1200-1000 cm-1 with first derivative treatment was chosen for FFA prediction, which yields a determination coefficients (R2) value of 0.9414, the satisfactory results of root mean square error of calibration (RMSEC) of 1.45 and root mean square error of prediction (RMSEP) value of 4.77, respectively. It can be concluded that FTIR spectroscopy combined with multivariate calibration (PCR) can be used as alternative method as it’s a rapid method and reduced volume of chemical solvent.

Keywords: Pangasius hypophthalmus oil, FTIR spectroscopy, free fatty acid, Principal Component Regression
ABSTRACT

Background: *Phyllanthus acidus* (Euphorbiaceae) is commonly known as cermai in Malaysia. This plant has been traditionally used in the treatment of inflammatory and oxidative stress related disorders. Objective: Despite its traditionally uses, the metabolites presented and bioactivities of *P. acidus* have inconsistency results. Therefore, this study was aimed to evaluate antioxidant, α-glucosidase- and nitric oxide (NO) inhibition activities of *P. acidus* leaves extracted with various ethanol ratios (0, 50 and 100 %) and to profile the chemical constituents in the active extract using tandem mass spectrometry (UHPLC-MS/MS).

Methods: Antioxidant activity was determined by TPC, DPPH and NO scavenging assays. The antidiabetic was evaluated through inhibition of α-glucosidase enzyme while the in vitro anti-inflammatory activity was evaluated using RAW 264.7 cells stimulated with LPS and IFN-γ and Griess method was employed to determine NO concentration. The UHPLC-MS/MS was utilized to identify the potential metabolites present in the active extract.

Results: The results showed that 50% ethanol was the most active extract with the highest TPC at 33.20 mg GAE/g extract, 40.45 % of DPPH, and moderate NO-scavenging and -inhibitory activities (IC\(_{50}\) = 158.17 and 180.06 µg/mL). It also exhibited the lowest IC\(_{50}\) value for α-glucosidase at 1.53 µg/mL. The UHPLC-MS/MS analysis showed the tentative identification of 30 metabolites, including derivatives of quercetin, kaempferol, epicatechin, coumaric, and cinnamic acids.

Conclusion: The extracts of *P. acidus* leaves that possessed antioxidant, α-glucosidase and NO inhibitory activities with beneficial metabolites that could be potential candidates for the treatment of diabetes and inflammation associated diseases.

Keywords: *Phyllanthus acidus*, leaves, antioxidant, anti-inflammatory, α-glucosidase.
Solid State Characteristics of Sublingual Tablet for Vaccine Formulation as Function of Cellulose Derivative Matrix and Oligosaccharide

Helmy Yusuf, Raditya Nugraheni, Maria Lucia Ardhani DL, Muh Agus Syamsur Rizal

Department of Pharmaceutics, Faculty of Pharmacy, Universitas Airlangga
Jl. Dharmawangsa Dalam 60286, Surabaya, Indonesia

*Presenting author
helmy-yusuf@ff.unair.ac.id

ABSTRACT

The present study describes the effect of cellulose derivative matrix and oligosaccharide on solid states characteristics of cationic dimethyl-dioctadecylammonium (DDA)-based liposomes in form of sublingual tablet for vaccine. Cellulose derivative matrix has been widely used in solid dispersion to prevent crystallization of entrapped drugs and/or aggregation. It increases mass and compact matrices of resultant dried cake. The results showed that cellulose matrix and oligosaccharide, regardless their concentration successfully maintained the intact structure of dried product. It prevented collapse as formulation that do not incorporated both excipients experienced massive collapse and shrinkage. Crystalline-forming properties were found using DSC, XRD and SEM. However, DSC data showed crystallization at very high temperature (>160°C), meaning that phase separation is avoidable at lower temperatures. The study revealed prospective advantages of using combination of both excipients in development of sublingual tablet of dried–DDA liposomal formulations for vaccine.

Keywords: DDA, Liposomes, cellulose matrix, oligosaccharide, sublingual tablet, vaccine

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In Vitro Dissolution Test of Bromelain Isolated from Pineapple Core Encapsulated in Hydrogel Semi-IPN Chitosan Methyl Cellulose

Ahmad Aly Irfan1*, Adinda Azkia1, Achmad Buhori1, HegiAdi Prabowo1, Elmira Vania1, Nathasya Humaira1 and Siswati Setiasih1

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, West Java, Indonesia

*Presenting author
Email: ahmad.aly@sci.ui.ac.id, Phone: +62 85770251529

ABSTRACT

Background: Bromelain is an enzyme that breaks down protein molecules and is widely used in therapeutic applications as well as in cardiovascular diseases. One source of bromelain is pineapple core. However, in its application as an enzyme-based oral drug, its activity can be reduced due to interactions with stomach fluids.

Objective: The isolated bromelain from the pineapple core is encapsulated with a hydrogel semi-IPN chitosan methyl cellulose in order to control its release and to maintain its activity.

Methods: Isolation of bromelain has been done with several stages of fractionation with ammonium sulfate salt and dialysis. Subsequently, the hydrogel semi-IPN chitosan methyl cellulose was synthesized for post-loading bromelain encapsulation. The bromelain dissolution capability is evaluated in vitro at gastric pH and artificial pH environments. Furthermore, the hydrogel is characterized using FTIR and an optical microscope.

Results: The specific activity of bromelain obtained from each purification steps shows an increment. The coarse enzymes, the ammonium sulfate fraction and the dialysis fractions have specific activity value of 22.39 U/mg, 76.73 U/mg, and 111.72 U/mg each. Hydrogel semi-IPN chitosan methyl cellulose was used for encapsulation which has the value of crosslinking degree of 46.63% and swelling ratio of 397.59%. Meanwhile, post bromelain loading encapsulation efficiency is 89.47%. The dissolution test results show that proteolytic activity of bromelain can be maintained up to 2.83 U/mL in artificial pH environment. Along with this, the release rate of bromelain is relatively larger in the artificial intestinal environment (35.61%) than artificial gastric fluid (29.27%).

Conclusion: Bromelain can be encapsulated in hydrogel semi-IPN of methyl cellulose chitosan. Hydrogels can maintain proteolytic activity of bromelain until it reaches the artificial intestinal environment.

Keywords: Bromelain, semi-IPN chitosan methyl cellulose, encapsulation, dissolution, proteolytic activity.
Metabolite Profiling of Ethyl Acetate from *Marsilea crenata* Presl. using UPLC-QTOF-MS/MS

Burhan Ma’arif\(^1,2\) and Mangestuti Agil\(^3\)

\(^1\)Doctoral Program of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia; 
\(^2\)Department of Pharmacy, Faculty of Medical and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia; 
\(^3\)Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

*Presenting Author*  
Email: burhan.maarif@gmail.com, Phone: 081335555725

**ABSTRACT**

*Marsilea crenata* Presl. is a plant that widely used as traditional food in Surabaya, Indonesia. Although in some research it was known to have activity in bone formation, the existence of *M. crenata* in present day is very rare. The aim of this research was to determine the metabolite profile of ethyl acetate extract of *M. crenata* using UPLC-QTOF-MS/MS, which can be used as a reference for further research and utilization of *M. crenata*. Dried powder of *M. crenata* was extracted with *n*-hexane followed by ethyl acetate using ultrasonic assisted extraction (UAE) method. 100 ppm of ethyl acetate extract in DCM and methanol then injected 5 µl each into the UPLC-QTOF-MS/MS. The results in the form of total ion chromatograms (TIC) were analyzed by Masslynx4.1 software and showed 57 compounds in DCM and 64 compounds in methanol.
ABSTRACT

**Background:** *Marsilea crenata* Presl. is a typical plant in East Java which have been suggested to have health benefits. The research shown that *Marsilea crenata* Presl. have a potential therapeutic for some diseases.

**Objective:** The aim of this research was to determine 96% ethanol extract compound of *Marsilea crenata* Presl. using UPLC-QTOF-MS/MS.

**Methods:** *Marsilea crenata* Presl. was extracted with 96% ethanol using Ultrasonic Assisted Extraction method. 100 ppm extract was prepared and then 5 µL on UPLC-QTOF-MS/MS. Results of the UPLC-QTOF-MS/MS in the form of total ion chromatograms (TIC) and then analyzed by software Masslynx 4.1.

**Results:** Which shown in each equipment DCM blank 55 compound and methanol blank 65 compound.

**Conclusion:** This is the first report of the application of non-targeted metabolomics in *Marsilea crenata* Presl.

**Key words:** *Marsilea crenata* Presl., metabolite profiling, UPLC-QTOF-MS/MS.
Chemical Analysis of Red Ginger (Zingiber officinale var Roscoe) Essential Oil and Its Anti-biofilm Activity against Candida albicans

Tristia Rinanda¹*, Rizki Puji Isnanda¹, and Zulfitri³

¹Department of Microbiology, Faculty of Medicine, Syiah Kuala University, Aceh, Indonesia, 23115
²Department of Biology, Faculty of Medicine, Syiah Kuala University, Aceh, Indonesia, 23115

*Presenting author
E-mail: tristia.rinanda@unsyiah.ac.id, Phone: (62)81360365000

ABSTRACT

Background: Biofilm formation is one of the virulence factors of Candida albicans and it contributes to the development of resistance to various antifungal drugs. In order to combat the resistant microbes such as C. albicans, the discovery and development of antifungal substances must explore the anti-biofilm activity of substances extracted from traditional medicinal plants widely available in tropical countries such as Indonesia. One of the natural ingredients that can be developed is red ginger. This plant has been used empirically to treat various infectious diseases, including fungal infections.

Objective: The aim of this study was to determine the composition of chemical compounds from the essential oil of the red ginger planted in Bireun, Aceh and also the anti-biofilm activity of the essential oil against C. albicans isolate.

Methods: The chemical analysis of the essential oil was performed by GC-MS. Anti-biofilm activity was observed through biofilm inhibition and degradation activities which were determined by using microplate spectrophotometer. The data were analyzed using ANOVA test and Duncan’s post hoc test with 99% CI.

Results: The GC-MS results showed that the essential oil used in this study contained high monoterpenes (60.55%), which is dominated by e-citral/geranial (11.97%) and 1.8-cineole (15.10%). The highest sesquiterpenes derivative was α-curcumene (16.86%). The significant inhibition of C. albicans biofilm formation was obtained at a concentration of 0.5% and the biofilm degradation was obtained at a concentration of 0.125%.

Conclusion: The data indicate that high monoterpenoids-red ginger essential oil used in this study has performed significant anti-biofilm activity against C. albicans.

Keywords: Red ginger, essential oil, biofilm, Candida albicans
DAY 2-PARAREL ROOM 1
(Garuda Mukti)-
(OP 37-OP 44)
Effects of Catechins Isolate of *Uncaria gambir* Roxb on Burn Wound Healing in Male Rats

Muhammad Yanis Musdja¹*, Lina Elvita¹, Nursetyowati Rahayu¹

¹Department of Pharmacy, Faculty of Medicine and Health Sciences, State Islamic University, Syarif Hidayatullah, Jakarta 15412, Indonesia

*Presenting author
Email: myanis88@gmail.com, Phone: +62812816811971

ABSTRACT

**Background:** Indonesia is the largest *Uncaria gambir*, Roxb (gambir) producing country in the world, but its benefits as a medicinal plant has not been optimally developed. Traditionally, gambir plants have been widely used by the community as wound healers.

**Objective:** This study aims to determine the efficacy of catechin isolate as a healing burn on white rats male Sprague Dawley strain through observation of anatomical and histopathological pathology.

**Methods:** Catechins of gambir were isolated by using partition method with ethyl acetate solvent. Rats were made to suffer burns with Akhoondinasab method, et al. A total of 30 male white rats were divided into 5 groups, given daily test preparations. A total of 3 groups were given catechin isolates prepared by gel with variations of concentrations of 1%, 2% and 4%. One group was used as a comparison with a patent medicine for healing burns and one other group was used as a negative control. After administration of the dosage for 21 days, the pathology and histology changes were observed in the burns that occurred.

**Results:** Statistical analysis showed that gel isolate catechins of gambir with concentrations 2% showed the effect of decreasing the burn area and an increase in the percentage of healing of burns that do not differ significantly from positive controls (P<0.05). Histopathologic observations showed that gambir catechin isolates were able to reduce the number of inflammatory cells and increase neocapillary formation compared with the negative control group.

**Conclusion:** Catechin isolates of gambir with concentration 2 % can help the healing process of second degree of burns in the inflammatory phase and proliferation.

**Keywords:** *Uncariagambir*, Catechins isolate, burn wound healing, male rats
Antiproliferation Activity of Ethanol Extract and Fraction of Soursop Leaves (A. muricata L.) on MCF7 Breast Cancer Cells

Ummi Habibah¹*, Yuni Elsa Hadisaputri¹,², Eli Halimah¹, Mutakin¹, RizkyAbdulah¹, and Ajeng Diantini¹

¹Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang KM.21 Jatinangor, West Java 45363, Indonesia;
²Division of Biological Activity, Centre Laboratory, UniversitasPadjadjaran, Jl. Raya Bandung-Sumedang KM.21 Jatinangor, West Java 45363, Indonesia

*Presenting author
Email: habibahummi07@gmail.com, Phone: 081912903626

ABSTRACT

Background: Breast cancer is the second most common cancer that occurs to women in the whole world and the incidence rate has increased annually. So that it takes traditional medicine as a solution to be offered.

Objective: Soursop is a traditional medicinal plant that has been widely used by the public as an anticancer. However, the anticancer effect has not been widely studied.

Methods: In this research, the antiproliferation activity test of ethanol extract and soursop leaf fraction (A. muricata L.) to MCF7 breast cancer cells were examined to determine the activity of cytotoxic using MTS assay method and morphological apoptotic test of breast cancer cell MCF7 to see the death cell.

Results: The results obtained are ethanol extract, ethyl acetate fraction, n-hexane fraction, and water fraction of soursop leaf having IC₅₀ value of each 1.832 μg/mL; 1.676 μg/mL; 2.245 μg/mL; 3.677 μg/mL, so the extract and fractions of soursop leaves can be said to have strong cytotoxic level with yield of IC₅₀<100 μg/mL value. High cytotoxic activity of soursop leaves can be demonstrated in apoptotic morphological tests of cancer cell, where it appears that the membrane and nucleus of cells undergo apoptotic that characterized by the rupture and loss of the membrane and nucleus.

Conclusion: So, it can be said that soursop leaf is a medicinal plant that has a high activity and effectivity as an anticancer for breast cancer treatment.

Keywords: Soursop (A. muricata L.), breast cancer cell MCF7, MTS Assay, cytotoxicity,apoptotic morphology.
ABSTRACT

**Background:** In 2015 the health ministry of Indonesia discloses data on the incidence rate of cancer in Indonesia. These data show that the special region of Yogyakarta has the highest prevalence of breast cancer incidence in Indonesia. Percentage of breast cancer incidence reached 2.4%. These patients experienced many treatment failures due to discomfort. Therefore, needed efforts to find new therapy of prevention and treatment for provide more comfort. Traditionally people from Bangka Belitung was use rumput gong (Eriocaulon cinereum R.Br) to cancer therapy.

**Objective:** A cytotoxic activity of dichloromethane fraction and ethyl acetate fraction against cancerous cells MCF7.

**Methods:** Fractionation was done by Vacuum Liquid Chromatography method with ethyl acetate and dichloromethane as eluent. The study of cytotoxic activity was measured using MTT assay method.

**Results:** The cytotoxic activity fraction of ethyl acetate shows IC\textsubscript{50} of 318.61 µg/ml and fraction of Dichlorometane shows IC\textsubscript{50} of 443.52 µg/ml.

**Conclusion:** The results of cytotoxic activity from fraction ethyl acetate fraction has the best cytotoxic activity so that further research is needed to find the compounds responsible for the activity.

**Keywords:** breast cancer, Eriocauloncinereum R.Br, MCF7.
Chemoprevention Effect of *Curcuma aeruginosa* in DMBA-Induced Rat Cytokines Production

Asri Sulfianti¹*, Nur Hasanah², Agung Eru Wibowo¹, Kurnia Agustini¹, Sri Ningsih¹, I Made Artika²

¹Center of Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology, Indonesia 15311
²Department of Biochemistry, Bogor Agricultural University, Bogor, Indonesia 16680

*Presenting author
Email: asrisulfianti88@gmail.com, Phone: +628588126350

**ABSTRACT**

**Background:** The member of Zingiberaceae, *Curcuma aeruginosa* Roxb. (*C. aeruginosa*) has been recognized as traditional medicine. Recent study reported this rhizome has pharmacological activities in treating tumor. Unfortunately, little work has been done in this herb to know the effect of *C. aeruginosa* as chemo-preventive agent against cancer cells.

**Objective:** to investigate the influence of *C. aeruginosa* extracts as chemo-preventive agent in Wistar rat induced by 7,12 dimetilbenz (a) antrasena (DMBA) on IL-2 and IL12 cytokines production.

**Methods:** animals were allocated to six group (n = 8 female rats each) as follows. An untreated group was used as control. Three other groups (CA1, CA2, CA3) were sequentially treated with 3 *C. aeruginosa* extract doses (CA1: 40 mg/ 200 g BW; CA2: 80 mg/ 200 g BW; CA3: 120 mg/ 200 g BW), DMBA and *Phyllanthus niruri* L. as negative and positive control groups. Body weight were registered in whole week in experiment. *C. aeruginosa* doses was given respectively to all group, except normal groups since acclimatization over to the end of experimental (20 weeks). After 2 weeks of extracts are given, rats induced by DMBA for 1 weeks. Some animals of the treatment groups were killed to investigate the tumor multiplicity. The development of tumor was evaluated until the 20th week. Blood collecting was done by 4 times to collect the serum and evaluate the IL-2 and IL12 productions.

**Results:** The treated of 80 mg doses *C. aeruginosa* as chemo-preventive represented the potential dose to decrease the tumor incidence and tumor multiplicity in this animal rat models. The doses also escalate the IL-12 production after DMBA induction until the end of experiment, tough did not significantly influence the IL-2 production.

**Conclusion:** *C. aeruginosa* as chemo-preventive agents in cancer development proven eliminate the cancer cells start from tumor development until metastatic phase.

**Keywords:** chemoprevention, *C. aeruginosa*, DMBA, wistar, cytokine.
Wound healing activity of Plantago major extract and its chemical compound on hyperglycemic rats

Kartini¹*, Ridho Islamie², Christina Cicilia Rambing¹, Alfisyah¹, Icha Bella Widya Siska Rahayu¹

¹Department of Pharmaceutical Biology, Faculty of Pharmacy University of Surabaya
²Department of Clinical and Community of Pharmacy, Faculty of Pharmacy University of Surabaya, Surabaya 60293, Indonesia

*Presenting author
Email: kartini@staff.ubaya.ac.id, Phone: +6281233927373

ABSTRACT

Background: Impaired wound healing in diabetic patients is a serious complication leading to amputation and even death. Proper diabetic wound management is required to improve the quality of life of diabetic patients. Plantago major (plantain) has been used empirically for wound healing. One of its chemical compounds, oleanolic acid (OA), has been studied on non-hyperglycemic wound.

Objective: This study was conducted to determine the wound healing activity of plantain and OA on hyperglycemic rats, as a model for diabetic wound.

Methods: A total of 40 hyperglycemic male rats (Wistar) were divided into 8 groups, and subsequently treated with gel of ethanol extract of P. major leaves and roots, OA, gel base, and bioplacenton®. Drugs were applied topically on animals’ wounds induced on the dorsal part, once a day for 21 days. Wound healing activity was evaluated based on the percentage of wound closure and wound healing time.

Results: The results showed that the gel of plantain leaves and roots extracts as well as OA were able to increase the percentage of wound closure (100%) compared to the negative control (83%). Moreover, gel of Plantain and OA accelerated wound healing time (15.8 and 10 days, respectively) compared to the negative control (24.4 days).

Conclusion: Plantain extracts and OA can be used as drug candidates for diabetic wound healing.

Keywords: Plantago major, oleanolic acid, diabetic wound, wound closure, healing time
Subfractionation of Ethyl Acetate Fraction of Mengkudu Fruit (*Morinda citrifolia* Linn.) and Antidiabetic Activity by Glucose Tolerance Method on Mice

Shintia Lintang Charisma*, Yasmiwar Susilawati2, and Ahmad Muhtadi3

1 Biological Pharmacy Department, Faculty of Pharmacy, Universitas Padjadjaran Jl. Raya Bandung Sumedang Km.21 Jatinangor, West Java, Indonesia
2 Biological Pharmacy Department, Faculty of Pharmacy, Universitas Padjadjaran Jl. Raya Bandung Sumedang Km.21 Jatinangor, West Java, Indonesia
3 Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran Jl. Raya Bandung Sumedang Km.21 Jatinangor, West Java, Indonesia

*Presenting author

Email: shintia16001@gmail.com, Phone: +6285846560177

**ABSTRACT**

**Background:** Mengkudu (*Morinda citrifolia* Linn.) fruit is one of the medicinal plant that contains alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, amino acids, saponnins, steroids, tannins, antraquinone and terpenoids. Traditionally, mengkudu (*Morinda citrifolia* Linn.) fruit has been used for antidiabetic effect. Previous research showed that ethyl acetate fraction gave significant activity (54.29 %) at dose 1200 mg/kg body weight on male wistar rats.

**Objective:** The aims of this study were: (a). To separate ethyl acetate fraction from mengkudu (*Morinda citrifolia* Linn.) fruit by Vacuum Liquid Chromatography method. (b). To study antidiabetic activities from mengkudu (*Morinda citrifolia* Linn.) fruit subfractions on male white mice by glucose tolerance method.

**Methods:** This research used separation guided by activity method. The ethyl acetate fraction separated by Vacuum Column Chromatography and gave five subfractions (Ds II-A, Ds II-B, Ds II-C, Ds II-D and Ds II-E). The five subfractions then tested the antidiabetic activity at a dose of 150 mg/kg body weight on male mice by glucose tolerance method. Blood glucose level measure at 30, 50, 90, 120 and 150 minutes after administration of subfractions.

**Results:** Ethyl acetate subfraction from mengkudu (*Morinda citrifolia* Linn.) fruit (Ds II-A, Ds II-B, Ds II-C, Ds II-D and Ds II-E) showed antidiabetic activity with percentage of relative glucose blood level (P%) at minute to 150 were 39.11%, 52.85%, 35.31% 43.55%, and 33.78% respectively.

**Conclusion:** The ethyl acetate subfraction of mengkudu (*Morinda citrifolia* Linn.) fruit showed potent antidiabetic activities that Ds II-B subfraction obtained the highest activity.

**Keywords:** Subfractionation, *Morinda citrifolia* Linn., Antidiabetic, Glucose tolerance method
In Vitro Anti-HIV Activity of Ethanol Extract from Gandarusa

(Justicia gendarussa Burm. f) Leaves

Ni Putu Ermi Hikmawanti¹, Prihartini Widiyanti²,³*, Bambang Prajogo EW⁴

¹Department of Pharmacy, Faculty of Pharmacy and Science, Universitas Muhammadiyah
²Faculty of Science and Technology, Universitas Airlangga, Indonesia
³Institute of Tropical Disease, Universitas Airlangga, Indonesia
⁴Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Indonesia

*Corresponding author.
Email address: pwidiyanti@fst.unair.ac.id

ABSTRACT

Justicia gendarussa Burm. f (Acanthaceae) is one of Indonesian medicinal plants. The present study was designed to evaluate anti-HIV activity of 70% fractionated-ethanol extract (with releasing alkaloids) and 70% ethanol extract (without releasing alkaloids) of J. gendarussa leaves on in vitro HIV-infected of MOLT-4 cells. The effect of the extracts in inhibiting viral replication and fusion process on acute HIV infection was identified and measured through syncytia formation assay. In addition, effect of the extracts on HIV p24 antigen was evaluated in a supernatant culture using HIV-1 p24 ELISA kit. It was found that 70% fractionated-ethanol extract and 70% ethanol extract of J. gendarussa leaves were significantly inhibited of HIV replication by inhibition of syncytia formation, where the 50% effective concentration (EC₅₀) values of the 70% fractionated-ethanol extract and 70% ethanol extract are 70.5 µg/ml and 228.7 µg/ml, respectively. Both of the extracts were also significantly inhibited HIV replication by decreasing HIV p24 antigen level where the 50% effective concentration values of the 70% fractionated-ethanol extract and 70% ethanol extract are 88.8 µg/ml and 540.7 µg/ml, respectively. Moreover, it was found that 70% fractionated-ethanol extract of J. gendarussa leaves has anti-HIV activity since its 50% effective concentration values less than 100 µg/ml. It could be concluded that J. gendarussa is a useful resource that could be developed into a phyto-pharmaceutical product with in vitro anti-HIV activity.

Keywords: Anti-HIV, Justicia gendarussa, MOLT-4 cell, p24 antigen, Syncytia formation
Enzymatic Esterification Ethyl Ester Fatty Acid from Hydrolyzed Castor Oil and Its Oxidation Product as Emulsifier and Antimicrobial Compound Using Candida rugosa Lipase E.C.3.1.1.3

Annisa Khairani¹*, Sumi Hudiyono¹, and Sri Handayani¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, West Java, Indonesia

*Presenting author
Email: annisakhairani52@gmail.com, Phone: +6281297307149

ABSTRACT

Background: The aim of this study was to synthesis of fatty acid ethyl ester compound of hydrolyzed castor oil and its oxidation product using Candida rugosa lipase.

Objective: Both esterification products were expected to have antimicrobial activity against Staphylococcus epidermidis and Propionibacterium acnes and may act as emulsifiers.

Methods: Optimization of esterification reactions was done by varying the mole ratio between fatty acids to ethanol, ie 1:1, 1:2, 1:3, and 1:4. The esterification products were then characterized using FTIR. Conversion percentage was determined by titrimetric method, to calculate the amount of fatty acids that have been converted to ester. Emulsifier test was performed to determine the ability of ester product as emulsifier. The esterification products were also examined their antimicrobial activity using disc diffusion method.

Results: The highest conversion percentage for fatty acid ethyl ester and its oxidation product were 76 % and 72% respectively. Characterization using FTIR for both ester showed the absorption band of C=O ester functional group at wave number 1731.27 cm⁻¹ and 1732.15 cm⁻¹ respectively. The emulsifier test showed that both esters have ability to stabilize emulsion up to 24 hours for water-in-oil emulsion (w/o) type. Antimicrobial assay showed that both esters have antimicrobial activity against both bacteria.

Conclusion: Fatty acid ethyl ester and its oxidized product using Candida rugosa lipase was successfully synthesized. Both esterification products have emulsifiers and antimicrobial activity.

Keywords: Castor oil, fatty acid ethyl ester, oxidized fatty acid ethyl ester, lipase, emulsifier, antimicrobial.
DAY 2-PARAREL ROOM 2
(R.300)
(OP 45-OP 52)
Beneficial effect of supercritical carbon dioxide extracted (SC-CO\textsubscript{2}) dabai (\textit{Canarium odontophyllum}) pulp oil in hypercholesterolemia-induced SPF Sprague-Dawley rats.

Noor Atiqah Aizan Abdul Kadir\textsuperscript{1}, Azrina Azlan\textsuperscript{1,2}, Faridah Abas\textsuperscript{3}, and Intan Safinar Ismail\textsuperscript{4}

\textsuperscript{1}Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia;
\textsuperscript{2}Research Centre for Excellence for Nutrition and Noncommunicable Disease, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia;
\textsuperscript{3}Department of Food Sciences, Faculty of Food Science and Technology, 43400 UPM Serdang, Selangor, Malaysia;
\textsuperscript{4}Department of Chemistry, Faculty of Science, 43400 UPM Serdang, Selangor, Malaysia.

\*Presenting author
Email: atiqahaizan@yahoo.com Phone: +60 13 899 5409

ABSTRACT

Background: The demand for edible oil has been increasing with the growing of world population and consumers’ preference for vegetable oils over animal fats. Production of dabai (\textit{Canarium odontophyllum}) pulp oil by supercritical carbon dioxide extraction is still relatively new in Malaysia and plausible to be investigated.

Objective: This study aims to investigate the effect of supercritical carbon dioxide extracted (SC-CO\textsubscript{2}) dabai pulp oil (DPO) in hypercholesterolemia induced rats

Methods: Male specific pathogen free (SPF) Sprague-Dawley rats were fed with high cholesterol diet for 4 weeks to induce hypercholesterolemia. The hypercholesterolemic rats were subsequently subdivided into positive control group (PG), low dose group (LG), high dose group (HG), and statin group (SG). The LG and HG groups were administered with 0.5\% and 2\% of SC-CO\textsubscript{2}DPO, respectively for another 4 weeks. Changes in body weight and biochemistry profile were measured.

Results: Hypercholesterolemic rats showed elevation of body weight and significant increment in total cholesterol (TC) and LDL levels when compared with normal rats consumed normal basal diet (NG) (p<0.05). Paired-samples t-test revealed that LG group showed a significant reduction in TC, triglyceride and LDL levels (p<0.05) and 8.26\% increment in HDL level. Meanwhile, reducing trends in TC, triglyceride and LDL levels were detected in HG group. No significant differences in AST and ALT levels were detected in LG and HG groups when compared with NG group.

Conclusion: These findings demonstrated that SC-CO\textsubscript{2}DPO contained potential factors contributing to cholesterol lowering effect and may serve as specialty oils for health promotion and disease prevention.

Keywords: \textit{Canarium odontophyllum}, dabai pulp oil, hypercholesterolemia, supercritical carbon dioxide extraction
Callus Induction of *Sonchus arvensis* L. and Its Antiplasmodial Activity

Dwi Kusuma Wahyuni¹, Hery Purnobasuki¹, Eko Prasetyo Kuncoro¹, Tutik Sri Wahyuni², Wiwied Ekasari², and Edy Setiti Wida Utami¹

¹Department of Biology Faculty of Sciences and Technology, Universitas Airlangga, Surabaya 60115; ²Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

*Presenting author
Email: kusumaanwar@yahoo.com, Phone: 085852134111

**ABSTRACT**

The absence of a vaccine and the rampant resistance to almost all antimalarial drugs have accentuated the urgent need for new antimalarial drugs. The aims of this study were to establish an effective protocol for callus induction from leaf explant of *Sonchus arvensis* L. and to investigate its *in-vitro* antiplasmodial activity. The effect of growth regulator was investigated. Growth regulator effect was studied by using 1 mg/L of Indolyl-3-acetic acid (IAA), Indolyl-3-butiric acid (IBA), Naphthalene acetic acid (NAA), and 2,4-diclorophenoxy-acetic acid (2,4-D) alone or in combination with 0.5 mg/L 6-benzyladenin (BA). Combination of 1 mg/L 2,4-D and 0.5 mg/L BAP produced the highest callusing and had the best quality of callus. The times required for callusing is shortest. Callus of 4 weeks old was extracted by methanol. *In-vitro* antiplasmodial activity of callus extract was investigated to against *Plasmodium falciparum* strain 3D7 with IC₅₀=1.254 µg/ml for callus by 1 mg/l 2,4-D+ 0.5 mg/L BAP treatment and IC₅₀=0.343 µg/ml for callus by 1mg/L IAA treatment.

**Keywords**: *Sonchus arvensis* L. antiplasmodal, *Plasmodium falciparum*, callus, growth regulator, metanaol extract, Tempuyung.
Production of secondary metabolites *catechin* by *in vitro* cultures *Camellia sinensis* L.

Sutini¹*, Widiwurjani¹, Djoko Agus Purwanto², Wirdhatul Muslihatin³

¹Agrotechnology Department of Agriculture Faculty UPN "Veteran" Jawa Timur, Indonesia.
²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya.
³Biology Department, Faculty of Mathematic and Natural Sciences, Institut Teknologi Sepuluh Nopember, Indonesia.

*Presenting author
Email: tien_basuki@yahoo.com

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**ABSTRACT**

**Background:** catechin is one of the secondary metabolites in the *Camellia sinensis* L plant, which can also be produced through *in vitro* cultures. This product of *in vitro* culture can be improved by optimizing the culture media with the addition of precursors.

**Objective:** find a method of production of catechin secondary metabolites through *in vitro* cultures *Camellia sinensis* L.

**Methods:** (1) cutting leaves inoculation of *Camellia sinensis* L., on Murashige and Skoog media with growth regulator, (2) callus inoculation product multiplicated on new media composition same as inoculation condition, (3) callus multiplication product developed on new media that containing precursor, (4) callus growth testing and harvesting, (5) callus identification with qualitative and quantitative methods.

**Result:** callus cultures contain a catechin secondary metabolite that grows increased by the addition of precursors

**Conclusion:** Provided secondary metabolite catechin which applicated as anti oxidant candidate

**Keywords:** *Camellia sinensis* L, catechin, *in vitro* culture, precursors.
Effect of Growth Regulators Indole Butyric Acid (IBA) and 6- Benzyl Amino Purin (BAP) on Callus Induction of Piper betle L. var Nigra

Junairiah¹*, Artifa Rachmah¹, Y. Sri Wulan Manuhara¹, Ni’matuzahroh¹, Lilis Sulistyorini²

¹Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115;
²Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia

*Presenting author
Email: alip.jun1@gmail.com, Phone: +6281331312165

ABSTRACT

Background: Piper betle L. var Nigra contains secondary metabolites and has biological activity. Secondary metabolite production has been done conventionally. This research provides an alternative isolation of secondary metabolites through callus cultures.

Objective: The purpose of this research to determine the effect of the combination concentration of growth regulators IBA (Indole Butyric Acid) and BAP (6-Benzyl Amino Purine) on callus induction of black betel (Piper betle L. var Nigra).

Methods: This research used completely randomized design with 25 treatments and 6 replicates of each treatment, hence there were 150 experimental units. At this stage of callus culture was done by adding the growth regulators IBA and BAP into Murashige and Skoog (MS). The test results showed that plant growth regulators IBA and BAP in combination with different concentrations of influence on callus induction time, fresh weight and dry weight callus of Piper betle L. var Nigra.

Results: The results showed the fastest time of callus formation at IBA 2.0 mg/L and BAP 2.0 mg/L at 10 days. Fresh weight and dry weight of the highest in the IBA 2.0 mg/L and BAP 2.0 mg/L were 0.8507 grams and 0.0769 grams fresh weight to dry weight. The color of callus was white greenish with compact and friable texture.

Conclusion: Growth regulators IBA 2.0 mg/L and BAP 2.0 mg/L produce the fastest induction time, the highest fresh weight and dry weight.

Keywords: Callus, Piper betle L. var Nigra, IBA, BAP
A New Naturally Biisoflavonoid Compound Isolated from Sesbania grandiflora

Noviany1*, Sutopo Hadi1, Neny Purwitasari2, and Hasnah Osman3

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, 35145, Indonesia
2Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, University of Airlangga, Surabaya, 60286, Indonesia
3School of Chemical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia

*Corresponding author: noviany@fmipa.unila.ac.id

ABSTRACT

Some new naturally phenolic compounds have been successfully isolated from the ethyl acetate extract of Sesbania grandiflora roots and stem barks. In our previous paper, we isolated a new naturally occurring binaphtol compound from the root of S. grandiflora, as well as an arylbenzofurans type from the stem barks of the same plant. We report herein, another new phenolic constituent from the ethyl acetate extract of S. grandiflora root, identified as 3,4-trans-4-[(3S)-7,2′-dihydroxy-4′-methoxyisoflavan-6-yl]-7,2′-dihydroxy-4′-methoxyisoflavan. This compound was found for the first time as a new naturally biisoflavonoid with (4→6) inter-isoflavanyl linkage from the Leguminosae plant, particularly from S. grandiflora.

Keywords: biisoflavonoid, phenolic compound; Sesbania grandiflora
Study of Bioaccumulation and Depuration of Pb metal ions in Green Mussels (*Perna viridis*)

Riska Tamala, Budiawan, and Sri Handayani

1Deapartment of Chemistry, FMIPA Universitas Indonesia, Kampus UI Depok, 16424, Indonesia

*Presenting author

Email: riskatamala50@gmail.com, Phone: +628998375099

ABSTRACT

In this study, bioaccumulation and depuration studies of Pb in green mussels were performed. The bioaccumulation process was carried out using flowing water method for 7 days. The Pb ion concentration used was 1.225 ppm. During bioaccumulation, Pb contained in mussels was determined every 24 hours. Two depuration methods were applied in this study, flowing clean water method for 7 days and immersing in acid solution for 2 hours. Two variations of acid solutions used were acetic acid and citric acid with variations concentration of 0.75%; 1.5%; and 2.25%. Pb contained in mussels was analyzed using AAS. The research showed that the highest value of Pb contained was reach after 7 days exposure with concentration of 41.92 mg/kg and concentration factor (CF) value of 32.15 L/kg. The lowest content of Pb was reached after depuration by immersing in 2.25% citric acid for 2 hours. Pb content after depuration was 16.96 mg/kg with the decrease of Pb by 59.5%. Bioaccumulation ability was expressed by Concentration Factor (CF). Based on this experiment, green mussel can be classified as low category bioindicator biota for Pb accumulation.

Keywords: Green mussel, Depuration, Lead, Atomic Absorption Spectroscopy (AAS)
In Vitro Study of DNA Adduct 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) Formation Due to Bisphenol A (BPA) Exposure Based on Fenton-like Reaction

Nadia Nahla Karima*, Budiawan1, and Sri Handayani1

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia

*Presenting author
Email: nadia.nahla@ui.ac.id, Phone: +6285211604541

ABSTRACT

Bisphenol A (BPA) is a chemical widely used in food packaging materials that can migrate into food and cause cellular oxidative stress that contributes to radical formation. The formed radicals can attack the DNA causing oxidative damage and formed 8-OHdG compound as a biomarker of carcinogenic risk. In addition, exposure to heavy metals from the environment can also lead to excess levels of Cu (II) in the body that increase the amount of radical. The formed radicals can attack the DNA causing oxidative damage and formed 8-OHdG compound as a biomarker of carcinogenic risk. The study of 8-OHdG formation was performed in vitro by reacting Calf thymus DNA and DNA base 2-dG with Bisphenol A through Fenton-like reaction in the presence of Cu (II). The variations used were pH 7.4 and 8.4, temperature at 37˚C and 60˚, and incubation time for 7 and 12 hours. The DNA adduct 8-OHdG formed was analyzed using HPLC reverse-phase chromatography using UV detector at 254 nm. The eluent used in this experiment was a mixture of buffer phosphate pH 6.7 10 mM and methanol at ratio 90:10. The results of the analysis showed that 8-OHdG concentration increased due to BPA exposure and Fenton-like reaction. Incubation time and temperature rise gave synergistic effect in increasing of 8-OHdG concentration, while the increase in pH does not have a synergistic effect on the 8-OHdG concentration.

Keywords: 2'-deoxyguanosine, 8-OHdG, BPA, Fenton-like, Cu (II), DNA Adduct
A New Antibacterial Polyketide from the Endophytic Fungi \textit{Aspergillus fumigatiaffinis}

Antonius R. B. Ola

Department of Chemistry, Faculty of Science and Engineering, Nusa Cendana University, Jalan Adisucipto Penfui, 85001 Kupang, Indonesia

ABSTRACT

Chemical investigation of the endophytic fungus \textit{Aspergillus fumigatiaffinis}, isolated from \textit{Tribulus terestris}, yielded a new poliketide, (−) palitantin. The structure of the compound was established on the basis of extensive 1D and 2D NMR spectroscopy, as well as by high resolution mass spectrometry and by CD spectroscopy. (−) palitantin was tested for its antimicrobial activity against multi-resistant clinical isolates of \textit{Staphylococcus aureus} 25697 and \textit{Enterococcus faecalis}, a susceptible reference strain of \textit{S. aureus} ATCC 29213 and against \textit{Streptococcus pneumoniae} ATCC 49619. (−) palitantin strongly inhibited \textit{Enterococcus faecalis} UW 2689 and \textit{S. pneumoniae} ATCC 49619 growth with a MIC value of 64 µg/mL.

Keywords: \textit{Tribulus terestris}, Timor, palitantin, Endophytic fungus, Antibiotic
DAY 2-PARAREL ROOM 3 
(R.301)(OP 53-OP 60)
In Silico Approach to Explore Indonesia’s Local Plants in Searching for Anti-Breast Cancer through Hemopexin MMP9 Interaction

Jason Rhinehard Karamoy, Pandu Hariyono, and Maywan Hariono

1Drug Discovery Research Group, Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta 55284, Indonesia

*Presenting author
Email: mhariono@usd.ac.id, Phone: +6289506286901

ABSTRACT

Background: Cancer is the third killer disease in the world which has no drug been ideal to overcome its full health restoration. The most notable problems are a drug resistance as well as an adverse drug side effect. Breast cancer is the most prevalence case in female which still keeps searching for the best healing. Hemopexin (PEX9) is the domain of Matrix Metalloproteinase 9 (MMP9) which is currently attractive due to its more selective target rather than MMP’s catalytic domain in searching for anti-breast cancer.

Objective: In this present study, we perform virtual screening of PEX9 inhibitor from Indonesia’s Herbal Remedies Database as an effort to identify potential Indonesian Plants to be used as herbal breast cancer remedies.

Methods: The method is by using AutoDock Vina as the program to perform in silico molecular docking against crystal structure of PEX9 from protein data bank.

Results: The result selects 20 hits from 200 as the total number of compounds in the database calculating free energy of binding within -11.2 to -8.1 kcal/mol, giving insight understandings of its potential as drug for breast cancer. The plant with its chemical substance is *Pachyrhizus erosus* (L). Urb. and pachyrhizin, respectively, is proposed for PEX9 inhibitor due to the free energy of binding (-8.5 kcal/mol), molecular weight (336.299) and its non-toxic property.

Conclusion: Based on the in-silico study, one of the local plants which is potential to be further studied as anti-breast cancer via PEX9 interaction mechanism is Bengkuang (*Pachyrhizus erosus* (L). Urb.

Keywords: In Silico, Indonesia’s plants, virtual screening, PEX9, MMP9
Development of pyrrolylated-chalcones as a lead compound to anti-inflammatory drugs

Siti Munirah Mohd Faudzi¹,²*, Faridah Abas²,³, and Nordin H. Lajis²

¹Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia;
²Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia;
³Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

*Presenting author
Email: sitimunirah@upm.edu.my, Phone: +60389466797

ABSTRACT

Background: Upon inflammation stimulation, macrophages produced a variety of inflammatory mediators including nitric oxide (NO) and prostaglandin E₂ (PGE₂). NO is an important free radical in the regulation of physiological and pathophysiological mechanisms. However, overproduction of NO might contribute to the degenerative diseases including cancer. Meanwhile, PGE₂ is a product of cyclooxygenase (COX) pathway which helps in sustaining homeostatic functions and mediate pathogenic mechanisms and is well identified as the lipid mediator that responsible for inflammatory-related disorders if in excessive production, wherein may contributes to rheumatoid arthritis and cancer.

Objective: Identification of new potent anti-inflammatory molecules in a series of synthesized pyrrolylated-chalcones by inhibiting NO and PGE₂ productions.

Methods: A series of twenty-four pyrrolylated-chalcones were synthesized and assessed for their NO and PGE₂ suppression on IFN-γ/LPS-induced RAW 264.7 macrophage cells. Further, evaluation for its toxicity and therapeutic effects as anti-inflammatory agents was conducted in zebrafish embryos in vivo model. Simultaneously, the molecular docking study also been performed to predict their molecular interactions.

Results: Four analogues exhibited good inhibitory properties in both NO and PGE₂, in which compound 2 (3-(2,5-dimethoxyphenyl)-1-(1H-pyrrol-2-yl)prop-2-en-1-one) showed the highest inhibition with IC₅₀ values of 12.1 ± 1.5 µM and 0.5 ± 1.5 µM, respectively. The docking studies of compound 2 were performed on 6COX (NF-κB pathway) and 1JNK (MAPK pathway), and the results revealed its capability to target the activity of downstream inflammatory COX-2 and JNK mediators. Furthermore, the lethal concentration (LC₅₀) for the four chalcones were evaluated using fish embryo toxicity test (FET) and the results indicates the LC₅₀ of the most potent compounds 2 is 5.916 ppm. As the LC₅₀ value of compound 2 is neither too high nor too low, along with the great in vitro anti-inflammatory activities, therefore, compound 2 was selected for in vivo anti-inflammatory evaluation. Based on the observation, it was suggested that compound 2 has a therapeutic effect against the toxicity induced by LPS exposure in zebrafish embryos.

Conclusion: The result suggested that compounds 2 could be potential lead for further development as new anti-inflammatory agents. Further works will address on mechanistic study on NF-κB pathways of compound 2 in vitro and in vivo models.

Keywords: Pyrrolylated-chalcones, anti-inflammatory, PGE₂, molecular docking, toxicity, zebrafish embryos in vivo model
ABSTRACT

Cancer is a class of disease which causes about 15% of human deaths worldwide and it is expected to increase in the coming years. It shows that cancer is a major health problem, with approximately 1.45 million new cases of cancer being discovered each year. Curcumin is one of natural product that has been widely used as traditional medicine, exhibit various biological activities including anticancer. However, curcumin which consist of β-diketone group is relatively unstable and suffers from poor bioavailability due decomposition of the compound with physiological pH. Thus, mono-carbonyl curcumin compounds analogs were synthesized to improve their activity and bioavailability. Base catalyzed condensation of 4-piperidine hydrochloride with substituted benzaldehyde under reflux led the formation of mono-carbonyl curcumin. The synthesized curcumin was further reacted with benzosulphonyl chloride or ethyl chloroacetate by nucleophilic substitution of –NH group to afford desired compounds with excellent yield.

Keywords: Anticancer, curcumin, hybride compound, monocarboxyl curcumin.
ABSTRACT

Cinnamamides are compounds that have at least a phenyl ring, olefinic and an amide group. This compound has been reported to have various biological activities such as anti-depressant, anti-tumor, anti-epileptic, analgesic, anti-inflammatory and anti-microbial activities. In our study, we have synthesized some of the cinnammamide derivatives from ethyl p-methoxycinnamate (1), in which 1 was found as a major compound in the rhizome of *Kaempferia galanga* and has been reported to have anti-inflammatory activity. The microwave-assisted reaction of the 1 by using ethanolamine, diethanolamine, urea and dimethylformamide with NaOH has been successful to give N-(hydroxyethyl)-p-methoxycinnamamide (2), N,N-bis(hydroxyethyl)-p-methoxycinnamamide (3), p-methoxycinnamamide (4) and N,N-dimethylp-methoxycinnamamide (5), respectively. Evaluation of the in vitro anti-inflammatory activity by using Bovine Serum Albumin (BSA) Assay indicated that compound 1-5 have an anti-inflammatory property. Furthermore, evaluation of in vivo anti-inflammatory activity by using carrageenan-induced paw edema method indicated that compound 2 and 3 have the ability in a decrease of inflammation condition.

**Keyword:** Cinnamamide, anti-inflammatory, ethyl p-methoxycinnamate, microwave-assisted reaction.
ABSTRACT

Background: The aim of this study is to synthesize glycerol – castor oil fatty acid and glycerol - oleic acid esters using Candida rugosa lipase in n-hexane.

Objective: Both glycerol – castor oil fatty acid and glycerol – oleic esters expected to have emulsifier and antibacterial properties.

Methods: Molar ratio Fatty acid to glycerol used in esterification were varied from 1:1, 1:2, 1:3, and 1:4. Ester products were then analyzed using FTIR. Conversion percentage in esterification was conducted using titrimetric method. Emulsifier test was performed to observe the stability and emulsion type, using ester product as emulsifier. Antimicrobial assay was also conducted for both esterification product using disc diffusion method against Propionibacterium acne and Staphylococcus epidermidis.

Results: FTIR spectra for glycerol-castor oil fatty acid ester and glycerol oleic ester showed the absorption peak at wave number 1735.37 cm\(^{-1}\) and 1748.25 cm\(^{-1}\) respectively, which indicate the existence of C=O ester groups. The highest conversion percentage was reached at the molar ratio 1:4 with the value of 92.42% respectively for glycerol – castor oil fatty acid ester and 86.24% for glycerol oleic ester. The properties of the emulsifier in both esters are obtained by type of water in the oil. The antimicrobial assay showed that glycerol-castor oil fatty acid ester has antimicrobial activity, but glycerol-oleic esters did not show the antimicrobial activity.

Conclusion: Glycerol – castor oil fatty acid and glycerol – oleic esters were successfully synthesized using Candida rugosa lipase as catalyst. Both ester products can be used as emulsifier for water in oil emulsion type. Only glycerol-castor oil fatty acid ester has antimicrobial activity.

Keywords: Castor oil, glycerol-castor oil fatty acid ester, oleic, emulsifier, antibacterial, Candida rugosa
ABSTRACT

Background: The purpose of this study was to synthesize glycol – castor oil fatty acid and glycol – palmitic acid esters using Candida rugosa lipase as biocatalyst.

Objective: The ester products was expected to have emulsifier and antimicrobial properties.

Methods: Esterification was conducted by reacting fatty acid and glycol at 37 °C for 18 hours. The variation of mmol ratio fatty acid to glycol used were 1:1, 1:2, 1:3, and 1:4. The ester product was characterized using FTIR and the conversion percentage was determined by titrimetric method. Emulsifier test also performed to determine the ability of ester product as emulsifier. Antimicrobial assay was also conducted using disc diffusion method against Propionibacterium acne and Staphylococcus epidermidis.

Results: FTIR spectra for glycol – castor oil fatty acid and glycol – palmitic esters showed the absorption of C=O functional groups at wave numbers 1732.27 and 1741.88 cm⁻¹, respectively. The highest conversion percentage value for glycol – castor oil fatty acid and glycol – palmitic ester were 85% and 82%, respectively. The emulsifier test showed that both glycol – fatty acid ester have properties as emulsifiers. Antimicrobial assay showed that glycol – castor oil fatty acid ester have activity as antimicrobial against Propionibacterium acne and Staphylococcus epidermidis. However, glycol palmitic ester has no activity as an antimicrobial agent.

Conclusion: The glycol – castor oil fatty acid and glycol – palmitic esters were successfully synthesized enzymatically using Candida rugosa lipase. Both ester products have properties as emulsifiers, but only glycol-castor oil fatty acid ester has potential to be an antimicrobial compound.

Keywords: Castor oil, fatty acid glycol ester, palmitic acid, lipase, emulsifier, antimicrobial.
Cytotoxicity of Fraction of Ethyl Acetate and Dichloromethane from Ethyl Acetate Extract
Eriocaulon cinereum R. Br on T47D (Breast Cancer) and Vero (Normal Cell)

Dian Nida Salsabila1*, Asgar Purnama1, Yoga Febriana1, HadyAnshory1, Arde Toga Nugraha1

1Department of Pharmacy, Faculty of Mathematics and Science, Islamic University of Indonesia, Yogyakarta 55584, Indonesia

*Presenting author
Email: 15613076@students.uii.ac.id, Phone: +6289650785715

ABSTRACT

Background: The prevalence of Breast cancer was 25.5% of all cancers in women worldwide. Based on data from Ministry of Health Republic Indonesia in 2013 stated that breast cancer is one disease with a high prevalence of the percentage of events 2.4% in Yogyakarta. Traditionally, Bangka Belitung People was used Eriocaulon cinereum R.Br as an anticancer drug.

Objective: Aims of this study determined the potential of cytotoxicity of Fraction of Ethyl Acetate and Dichloromethane against T47D cell.

Methods: Fractionation was performed on ethyl acetate extract used Vacuum Liquid Chromatography (VLC) method. The solvents used dichloromethane and ethyl acetate. Evaluated of cytotoxic activity was test used MTT assay method.

Results: The fraction of dichloromethane had high activity as a chemopreventive agent with IC50 value of 131.921 µg/ml to T47D and 413.042 µg/ml cells to normal cell (Vero) whereas for ethyl acetate fraction had weaker activity as chemopreventive agent with IC50 value equal to 531.808 µg/ml to T47D and 679.114 µg/ml to normal cell (Vero). Phytochemical tests showed that the dichloromethane fraction contains phenolic, steroid and terpenoid compounds. While ethyl acetate fraction contains alkaloids, flavonoids, and terpenoids. These compounds had been demonstrated in previous studies to have activity on cancer cells through apoptotic mechanisms as well as proliferation.

Conclusion: The results show the excellent potential of this plant in killed breast cancer cells. Therefore, it is necessary to do deeper research to be able to determine the compounds that are responsible as anti-cancer.

Keywords: Breast cancer, Eriocauloncinereum R.Br, T47D, Vero
Antiviral activities of curcuma genus against hepatitis C virus

Tutik Sri Wahyuni\textsuperscript{1,2*}, Adita Ayu Permatasari\textsuperscript{2}, Humairoh Mahfud\textsuperscript{1}, Safaatul Laysa\textsuperscript{1}, M. Dzul Azmi\textsuperscript{1}, An Nisa Nur laila\textsuperscript{1}, Aty Widyawaruyanti\textsuperscript{1,2}, Achmad Fuad\textsuperscript{1,2}.

\textsuperscript{1}Department of Pharmacognocy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115; \textsuperscript{2}Institute of Tropical Disease, Universitas Airlangga Surabaya 60115

*Presenting author

Email: wahyuni.tutiksri@yahoo.com, tutik-s-w@ff.unair.ac.id; Phone :6281241435799

ABSTRACT

Background: Hepatitis C virus infection is worldwide public health problem. Even through the new agents shown to increase the sustain virology response however there are still many people could not access to the therapy due to the high cost. Moreover, the emerge issue of resistance and side effects presented the necessity to develop alternative treatment agents for hepatitis C virus infection. Plants with the genus of curcuma are popular among traditional medicine in the world including Indonesia. They have been used for many herb remedies and reported to possess many biological activities. Several plants from curcuma genus were known for treatment agent in liver disease and jaundice.

Objective: Our current study determines antiviral activities of \textit{Curcuma domestica}, \textit{Curcuma xanthoriza}, and \textit{Curcuma heyneana} against hepatitis C virus and further examine the mechanism of actions.

Methods: Plants material was extracted with ethanol. The tested extracts were dissolved in dimethyl sulfoxide and further diluted in serial concentrations with dubaco medium. Antiviral activity was performed by \textit{in vitro} culture cells. Huh 7.5it cells were cultured in 48 multiwall plate, then treated with the mixture of extract and virus JFH1. The effect of extracts in HCV life cycle were determine by mode of action analysis.

Results: Extract of \textit{Curcuma domestica}, \textit{Curcuma xanthoriza}, and \textit{Curcuma heyneana} revealed anti-hepatitis C activities with IC\textsubscript{50} value of 1.68 ± 0.05, 4.93 ± 0.42 and 5.49 ± 0.59, respectively. Mode of action analysis was demonstrated that all of extracts tested exhibit stronger activities in the entry step of HCV life cycle.

Conclusion: Those results suggested that \textit{Curcuma domestica}, \textit{Curcuma xanthoriza}, and \textit{Curcuma heyneana} possessed strong inhibition against hepatitis C virus therefore they may a good candidate for anti-HCV agents.

Keywords: Hepatitis C virus, \textit{Curcuma domestica}, \textit{Curcuma xanthoriza}, \textit{Curcuma heyneana}. 
DAY 2-PARAREL ROOM 4
(R.305)
(OP 61-OP 68)
Kinetic Studies of Purified Bromelain from Palembang Pineapple (Ananas comosus [L.]Merr) Core with Hydroxypatite and CM Sephadex C-50 Ion Exchange Chromatography

Nofa Rahayu Desi Putri¹*, Sumi Hudiyono¹ and Siswati Setiasih¹

¹Department of Chemistry, Faculty of Science, Universitas Indonesia, Depok, 16424, Indonesia;
*Presenting author
Email: nofarahayudp@gmail.com, Phone: +6281329255501

ABSTRACT

Bromelain is the pineapple aqueous extract that contains complex mixtures of proteases and non-protease components. These enzymes perform an important role in proteolytic modulation of the cellular matrix in numerous physiologic processes, including anti-inflammatory, anti-thrombotic and fibrinolytic functions. Here we purposed a new purification step to collect enzyme from pineapple. The purification of the enzyme from Ananas comosus was carried out by precipitation with varying concentration of acetone and followed by column chromatography using hydroxypatite ion exchange and CM sephadex C-50 resin. The enzyme activities were evaluated using casein as substrate. The highest specific activity of bromelain was gained from acetone fractination as 51.51 U/mg in the range of 50-80% saturation with purity level of enzyme as 11 times from its crude extract. The highest specific activity from last fraction which run on hydroxypatite ion-exchange column chromatography was 87.49 units/mg with purity level of enzyme resulted 19 times higher compared to the crude extract. Later on, the purification with CM sephadex C-50 resulted in increasing the specific activity to 200 U/mg with purity level of enzyme as 45 times higher from its crude extract. Hydrolysis of various casein concentration with purified bromelain was carried out at optimum reaction condition of pH 7.0 and 37ºC. The results obtained revealed the Km and Vmax value were 0.94% (w/v) and 0.023 U/min respectively. From the various purification steps that have been done, it can be observed the increasing of bromelain specific activity from each stage.

Keywords: Pineapple core, bromelain, specific activity, Michaelis-Menten constant, maximum reaction velocity.
Noodles ‘kremes’ that Enrichment Pure of *Moringa oleifera* Leaf as Functional Snack for Children at Insecurity area

Annis Catur Adi and Agnes Nanda Arimbi

Dept of Nutrition, Faculty of Public Health, Universitas Airlangga

**ABSTRACT**

Wild foods and under-utilized foods contribute significantly as the source of nutrients and bioactive components for rural and poor households at insecurity area. *Moringa oleifera* or in Indonesia known as “kelor” is one of wild plants which can be utilized as raw food materia. The purpose of this study was to analyze the level of preference, nutrition and bioactive contain in the noodle “kremes” that added pure of *Moringa* leaf. This study applied experimental study design using completely randomized design with six repetitions. Organoleptic with hedonict test and data concerning to organoleptic was processed using Kriskal-Wallis test to determine the difference among formulas (F0= 0%, F1=10% and F3= 15%) and was described descriptively.

Study found that the acceptance of *Moringa oleifera* as part of igerdient of noodle “kremes”. An organoleptic test the consisted of 30 untrained panelists showed that Formula F1 (added 10%) was the most the preference level of noodle kremes. There was difference in the level of aroma (p=0,009) and taste (p=0,04), but texture and colour no big difference between the three of formulas noodle. Noodle “kremes” that added with *Moringa* oleifera, beside containts of enough rich nutrients (protein, vitamin A and C), also contains terpenoid, polyphenol and flavonoid. Noddles “kremes that enrichment with pure of *Moringa oleifera* leaf can be used as functional snack for children at insecurity area

**Keywords:** Noodle, Funtional snack, *Moringa oleifera*, Wild plants
Formulation and Evaluation of Piper betle Linn Extract-Chitosan Spray Gel Spray as Wound Healing

Retno Sari*, Lintang Indra Sukmawati, Esti Hendradi

Faculty of Pharmacy, Airlangga University, Surabaya

*Presenting Author
Email: retno-s@ff.unair.ac.id, Phone: 081565855657

ABSTRACT

Chitosan is a polysaccharide consisting of glucosamine and N-acetylglucosamine which can be used as wound healing. Piper betle L. extract which has activity as antibacterial can be used to improve the effectiveness of chitosan as wound healing agent. The spray gel was chosen since it can reduce the pain when administered to the wound and easily use. The spray gel was prepared using chitosan concentration 0.5% to 1.0%. The spray gel was evaluated for its organoleptic, viscosity, spreadability, drying time, pH and stability. The acceptibility test was also performed. The results showed that the chitosan concentration affected the viscosity, pH, spreadability and drying time. The result of stability test showed that the viscosity was decreased, pH was stable, the spreadability and the drying time was increased. The spray gel with lowest chitosan concentration had highest acceptability among the others.

Keywords: chitosan, Piper betle L, spray gel, wound healing
Kinetik Study of Bromelain from The Core of Pineapple Extracts (*Ananas comosus*) and Purification by using Ion Exchange Column Chromatography DEAE-Sepharose

Nita Magfirah Ilyas, Siswati Setiasih and Sumi Hudiyono

*Departement of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok 16424, Indonesia*

*Presenting Author*

Email: nita.chemist12@gmail.com, Phone: 082393492219

**ABSTRACT**

The aim of this research was to isolate and purify bromelain from core extract of pineapple (*Ananas comosus*) through fractionation using ammonium sulfate followed by dialysis and then purification using ion exchange column chromatography DEAE-Sepharose. The fraction of bromelain obtained from each purification step showed an increase in specific activity compared to crude extract. Fractionation of crude enzyme bromelain with ammonium sulfate produces highest specific activity on ammonium sulfate 30-60% fraction (fraction 2) 260.042 U/mg with purify level 2.548-fold compared to crude extract. After dialysis, the bromelain fraction showed an increase in specific activity (381.287 U/mg) with purify level 3.737-fold compared to crude extract. The bromelain fraction after purification by using ion exchange column chromatography produces highest specific activity on DEAE chromatography fraction 3 (fraction number 64-86) 500 U/mg with purify level 4.901-fold compared to crude extract. Furthermore, bromelain fraction with the highest specific activity from ion exchange chromatography step was then determined the kinetic parameter such as Michaelis-Menten constant (Km) and maximal velocity (Vmax) of bromelain reaction. After plotting the Lineweaver-Burk graphic obtained the value of Michaelis-Menten constant (Km) 0.84 and maximal velocity (Vmax) of bromelain reaction 0.043 U/min.

**Keywords:** *Ananas comosus*, pineapple core, bromelain, purification, chromatography, kinetic study
POSTER PRESENTATION
(R.300) (PP 1-PP 99)
Protective activity of Chitosan Nanoparticle against Cadmium Chloride induced gastric toxicity in Rat

Giftania Wardani Sudjarwo

Department of Pharmachy Biology, Faculty of Pharmachy, Hang Tuah University Surabaya

Email: giftania.wardani@hangtuah.ac.id

ABSTRACT

Cadmium is one of the most toxic metals, producing gastric toxicity in animals and humans. Oxidative stress reported to play an important role in cadmium chloride induced gastric injury. This study was carried to investigate the role of chitosan nanoparticle in protecting against cadmium chloride-induced gastric toxicity in rat. The sample used 50 male rats were divided into 5 groups: negative control (Rats were given daily with aquadest); positive control (Rats were given daily with cadmium chloride 5 mg/kg BW orally once in a day for 28 days) and the treatment group (Rats were given the chitosan nanoparticle 150 mg; 300 mg; 500 mg/kg BW orally once in a day for 32 days and on 4 day, were given cadmium chloride 5 mg/kg BW one hour after the chitosan nanoparticle administration for 28 days). On day 32, the rats were sacrificed, and gastric tissues were collected to evaluate the ulcers and to measure Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). The gastric tissues also were subjected to histological evaluations. The results showed that, Oral administration of cadmium chloride 5 mg/kg BW for 28 days significant induced gastric mucosal hemorrhagic lesions and significant decrease in SOD and GPx in rats. Histological analysis shown that cadmium also induced necrosis of gastric mucosal epithelial cell in the negative control group. Treatment with the chitosan nanoparticle 600 mg/kg BW but not 150 mg/kg BW and 300 mg/kg BW significantly (P< 0.05) improved gastric injury and decreased MDA levels as compared to positive control group. Treatment with the chitosan nanoparticle 600 mg/kg BW also significant increase in SOD and GPx as compared to positive control group. The rats pretreated with chitosan nanoparticle 600 mg/kg BW demonstrated significantly improved necrosis of gastric mucosal epithelial cell. From the results of this study concluded that the chitosan nanoparticle could be a potent natural product provide a promising gastroprotective effect against cadmium chloride induced gastric toxicity in rats.

Keywords: Chitosan nanoparticle, Cadmium chloride, Gastric injury, MDA, SOD, GPx

PP1
Purification and Characterization of E. Coli β-Glucuronidase from Plectranthusamboinicus (Spreng.)

Michael Russelle Alvarez1*, Punsaldulam Dashnyam2, Nitish Verma2, Chun-Hung Lin2, Francisco Heralde III1

1College of Medicine, University of the Philippines Manila, Manila City, Philippines; 2Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

*Presenting author
Email: russellealvarez@gmail.com, Phone: 639171404862

ABSTRACT

Backgrounds: An approach to alleviating CPT-11, an anticancer drug for colorectal cancer, toxicity is by discovering selective nontoxic (against bacteria) inhibitors of bacterial β-glucuronidase (GUS).

Objectives: In this study, the GUS inhibitory activity of Plectranthusamboinicus, locally known as oregano, against E. coli, an ubiquitous bacteria found in the human gut, was determined.

Methods: Bioassay-guided purification of P. amboinicus leaf chloroform extract was performed using gravity column, flash chromatography, C18-HPLC, and crystallization. The enzyme assay used to screen β-glucuronidase inhibitory activity was the 4-methylumbelliferyl glucuronide (4-MUG) assay, using E. coli recombinant GUS and human recombinant GUS. After each succeeding purification step, phytochemical staining was performed using TLC spray tests.

Results: After multiple purification steps, several fractions were obtained having significant inhibitory activity (IC50): PA CHCl3-5-2-3-1 (3.087 µg/mL), PA CHCl3-5-2-3-5 (1.537 µg/mL), PA CHCl3-5-2-3-6 (3.852 µg/mL), PA CHCl3-5-2-3-5-ACN (12.93 µg/mL), PA CHCl3-5-4-3-wash (16.24 µg/mL), PA CHCl3-5-6-3-3 (36.38 µg/mL), PA CHCl3-5-6-3-4 (5.414 µg/mL), and PA CHCl3-5-7-ACN-11.5 (4.422 µg/mL). These active fractions were also tested against the human GUS for selectivity; by comparing the average percent inhibition of the fractions against the human and E. coli enzymes and using subsequent t-tests, we found that these fractions indeed had less activity against the human enzyme (p-value<0.0001, α=0.05). Through phytochemical tests, the active fractions were found to be mostly positive for the unsaturation and aromatic groups (Iodine stain), sterols and steroids (sulfuric acid-methanol stain) and cholesterol, cholesterol esters and terpenoids (Liebermann-Burchard stain).

Conclusion: This study shows the β-glucuronidase inhibitory activity of P. amboinicus. Currently, GC-MS metabolite profiling is being performed to identify the compounds present in each fraction. This, coupled with in silico docking onto E. coli and human β-glucuronidase, will be done to characterize the interactions of each compound to the enzyme.

Keywords: β-glucuronidase, Plectranthusamboinicus, E. coli, purification
Antihyperglycemia Effect of Tomato Juice (*Solanum lycopersicum*) on White Rats (*Rattus norvegicus*) Sprague dawley Strain with Induced Alloxan

Hanifa Irma Ika Situmorang¹, Anita Lidesna Shinta Amat², Kartini Lidia³

¹Medical Faculty of Nusa Cendana University,  
²Department of Biochemistry, Medical Faculty of Nusa Cendana University,  
³Department of Pharmacology and Pharmacotherapy Medical Faculty of Nusa Cendana University

*Presenting author  
Email: anita.lidesna.shinta@gmail.com, Phone: 081219144885

ABSTRACT

**Background:** Hyperglycemia is an abnormal raising of blood glucose level, characterise diabetes melitus. Tomato products are a dietary source of natural antioxidants, especially lycopene, which has an ability to improve beta pancreas cell with the result that increase secretion of insulin and maintain blood glucose at normal level.

**Objective:** This research was conducted to determine antihyperglycemia effect of tomato juice (*Solanum lycopersicum*) on white rats (*Rattus norvegicus*) Sprague dawley strain with induced alloxan.

**Method:** This research used experimental method with true experimental design - randomized pre and post test controlled group. Adult *Sprague dawley* rats (15 rats) were randomly grouped in several experimental group. Positive control group (given glibenclamid 0.9 mg/200 g BW), negative control (given aquadest), and three groups with different doses of juice (dose 1 = 2.25 ml/200 g BW, dose 2 = 4.5 ml/200 gBW, dose 3 = 9 ml/200 gBW). The groups were pre-tested to find out the initial state among the groups. Then after being given various treatments proceeded by doing post-test on the groups to observe the effect of giving various treatments to the groups.

**Results:** The result shows that there is significant antihyperglycemia effects of tomato juice dose 1 (2.25ml/200 g BW) with probability value of < 0.05. The percentage decrease of blood glucose level was 55.3% in low-dose group, 20.8% in medium-dose group, 18.8% in high-dose group.

**Conclusions:** Tomato juice (*Solanum lycopersicum*) has antihyperglycemia effects.

**Keywords:** *Solanum lycopersicum* fruit, Hyperglycemia, Lycopene, Sprague dawley
Artocarpus altilis leaves activity in inhibiting α-Amylase Enzyme as Oral Antidiabetic Drug Candidate

Lusi Putri Dwita¹*, Vivi Anggia¹, and Tri Dewi Prasetyatuti¹

¹Faculty of Pharmacy and Science, Universitas Prof. DR. HAMKA (UHAMKA), East Jakarta13460, Indonesia

*Presenting author
Email: lusi_putridwita@uhamka.ac.id, Phone: +6281322408072

ABSTRACT

Background: The search for effective antidiabetic drugs remains to be a focus of research in the world. Artocarpus have been used as traditional medicine in South-East Asia. One of the species, Artocarpus altilis has been known in Indonesia as traditional treatment for diabetic.

Objective: This research aimed to determine the activity of ethanol extract 70% of Artocarpus altilis leaves in inhibiting α-amylase.

Methods: Leaves were extracted by maceration method, followed by phytochemical screening and quercetin identification using LC-MS method. Inhibition of α-amylase was measured using UV-Vis spectrophotometer at 524 nm wavelength.

Results: Results showed a potential activity of 70% of Artocarpus altilis leaves ethanol extract against α-amylase with IC₅₀ value of 156.04 μg/ml and 0.27 times relative potential compared to acarbose.

Conclusion: Artocarpus altilis showed a potential activity in inhibiting α-Amylase.

Keywords: Artocarpus altilis, α-Amylase, antidiabetic
In Vitro Antibacterial Activity Test of Jackfruit (Artocarpus heterophyllus Lam.) Leaf Extract against Methicillin-Resistant Staphylococcus aureus (MRSA)

Ika Ningsih¹, Dyah Ayu Rosalinda², Ariyani Kiranasari¹, Beti Ernawati Dewi¹, Fithriyah Sjatha¹

¹Microbiology Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur no. 16 Jakarta 10320, Indonesia; ²Undergraduate Student, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur no. 16 Jakarta 10320, Indonesia

*Presenting author
Email: fithriyah31@ui.ac.id, Phone: 021 31922850, 021 310080

ABSTRACT

Introduction: Infectious diseases are still a public health problem in Indonesia. Methicillin Resistant Staphylococcus aureus (MRSA) is one of bacteria causing infections that is a concern because of the nature of resistance to various beta-lactam class of antibiotics. Vancomycin is still the drug of choice for MRSA infections but in recent years research shows that it has been found strains of MRSA that decreased sensitivity to vancomycin. Therefore, it is necessary to find an alternative antibiotic for the treatment of MRSA infections.

Objective: This study aims to determine the antibacterial activity of jackfruit (Artocarpus heterophyllus Lam.) leaf extract against MRSA by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

Method: The study was conducted using in-vitro test with broth macrodilution method. Jackfruit (Artocarpus heterophyllus Lam.) leaf extract were used in various concentration of 1280 μg/mL, 640 μg/mL, 320 μg/mL, 160 μg/mL, 80 μg/mL, 40 μg/mL, 20 μg/mL, 10 μg/mL, 5 μg/mL, dan 2.5 μg/mL, 1.25 μg/mL, and 0.625 μg/mL.

Result: MIC of jackfruit leaf extract against MRSA was found at a concentration of 320 μg/mL showed by a clear solution in the tube with extract concentration of 320 μg/mL, 640 μg/mL, and 1280 μg/mL. MBC of jackfruit leaf extract against MRSA was found at a concentration of 1280 μg/mL because there was no growth of MRSA colonies on Mueller-Hinton agar.

Conclusion: From this study showed that jackfruit (Artocarpus heterophyllus Lam.) leaf extract has anti-bacterial activity against MRSA in-vitro.

Keywords: Antibacterial activity, jackfruit (Artocarpus heterophyllus Lam.) leaf extract, Methicillin-Resistant Staphylococcus aureus
ABSTRACT

Backgrounds: In this current era, there is an increase in the total number of infectious diseases worldwide, which is to treat infectious diseases, doctors often prescribe antibiotics to patients. However, in daily practice, there is a lot of misuse of antibiotics for treating infections such as using improper antibiotics or lack of patient’s compliance which eventually leads to the emergence of antibiotic resistant microbes. Given its cultural values, abundance of natural resources, Indonesia must be on the forefront of research on alternative medicine, especially in finding new antibacterial candidates resourced from endemic Indonesian plants.

Objective: The objective of this research is to assess the antibacterial activities of several Indonesian endemic plants extract against Staphylococcus epidermidis, Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA) in-vitro.

Methods: Several plants extracts were tested its antibacterial activity in several concentration through microdilution methods to get MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) value and through agar diffusion to get inhibition zone, compared to ciprofloxacin antibiotic as positive control.

Results: Extract from Piper betle have a higher inhibition zone compare to the ciprofloxacin antibiotic control. Meanwhile, Syzygium aromaticum extract was found to have antimicrobial properties against MRSA (MIC: 0.30906%, MBC: 0.30906%) and Staphylococcus aureus (MIC: 0.7813%, MBC: 0.7813%).

Conclusion. Extract from Piper betle and Syzygium aromaticum were found to have a good antibacterial activity which will be further analyzed for extract purification and bacterial inhibition mechanism.

Keywords: antibacterial, extract, plant
Antiviral Activity of *Cynometra ramiflora* Linn Leaves Extract Against Replication of Dengue Virus Serotype 2 on Huh 7.5 Cell In Vitro

Amry Irsyada Yusuf¹, Beti Ernawati Dewi², Fithriyah Sjatha²*

¹Undergraduate Student, Faculty of Medicine, Universitas Indonesia;
²Microbiology Department, Faculty of Medicine, Universitas Indonesia, Jl. Pegangsaan Timur no. 16 Jakarta 10320

*Presenting author
Email: fithriyah31@ui.ac.id, Phone: 021 31922850, 021 310080

ABSTRACT

**Introduction:** Dengue hemorrhagic fever (DHF) remains a major health problem of world particularly in Indonesia due to high morality rate of it. Until now, there is no specific antiviral therapy for DENV yet and the treatment is still supportive. The extract of *Cynometra ramiflora* Linn leaves known to have some effects such as bactericide, analgesic, antiviral, anti-inflammation, and anti-allergy.

**Objectives:** The objective of this study is to assessed antiviral activity of *Cynometra ramiflora* Linn leaves extract against DENV-2 in-vitro.

**Methods:** The potency *Cynometra ramiflora* Linn leaves extract at concentration of 1.25 μg/ml, 2.5 μg/ml, 5 μg/ml, 10 μg/ml, dan 20 μg/ml as anti-viral dengue (DENV) was performed in vitro on Huh 7.5 cell infected by DENV-2 with MOI 0.5. Positive control in this research was Huh 7.5 cell infected by DENV-2, group of Huh 7.5 with dimethyl sulfoxide (DMSO) as negative control, and group of Huh 7.5 cell only as cell control. Each group was done in six repetitions. The inhibition rate of the extract to DENV replication was measured using foci-forming immunoassay.

**Results:** Statistically administration *Cynometra ramiflora* Linn leaves extract showed significant inhibition at each concentration (p < 0.05) compared with positive control. The inhibition rate was 36.06 %, 45.96 %, 47.35%, 55.94%, 62.70% at concentration of 1.25 μg/ml, 2.5 μg/ml, 5 μg/ml, 10 μg/ml, dan 20 μg/ml respectively.

**Conclusion:** The result of this study showed that extract of *Cynometra ramiflora* Linn leaves has potency as antiviral dengue.

**Keywords:** Antiviral; *Cynometra ramiflora* Linn; Dengue Virus; Huh 7.5 Cell
Antimalaria activity of ethanolic extract of *Macaranga gigantea* leaf and its major constituents

Muhammadina,2*, Yusnaidar1,2, Hilda Amanda2, Madyawati Latief2, Nurumawati Lase2, Anis Yohana Chaerunisaa3, Andreas Yoga Aditama4 and Josephine Elizabeth Siregar4

1Department of Chemistry Education, Faculty of Education, University of Jambi, Jambi, 36361 Indonesia; 2Department of Chemistry, Faculty of Science and Technology, University of Jambi, Jambi, 36361, Indonesia; 3Faculty of Pharmacy, Padjadjaran University, Jatinangor, 45363, Indonesia; 4Mitochondria and Infectious Diseases Laboratory, Eijkman Institute for Molecular Biology, Jakarta, 10430, Indonesia

*Presenting author
Email: muhaimin_73@yahoo.de, Phone: +6281394014688

ABSTRACT

**Background:** *Macaranga gigantea* has been used in traditional medicine as antimalaria remedy by various tribes in Jambi region, Indonesia.

**Objective:** This research aimed to study the activity of ethanolic leaf extract of *M. gigantea* and its major constituents on malaria parasites using *ex vivo* model.

**Methods:** This study was conducted by extraction of *M. gigantea* leaves using ethanol and isolation of its major constituents. The extract and isolate were tested *ex vivo* against *Plasmodium berghei* strain ANKA infected into Balb-C mice, and antiplasmodial activity were observed by calculating the parasitaemia percentage after 24 hours. Antiplasmodial activity expressed as decreased of parasitaemia levels and percent of inhibition. Chemical composition of ethanolic crude extract and isolate were tested by phytochemical screening. Structure of isolate was characterized using UV-Vis and FTIR spectrophotometry methods.

**Results:** The results revealed that ethanolic crude extract of *M. gigantea* leaves contained flavonoid, alkaloid, phenol, terpene, and steroid. Its *ex vivo* antiplasmodial activity showed percent inhibition of 92.1; 85.7; 64.1; 41.5 and 21.7% at concentrations of 300, 100, 30, 10 and 3 μg/mL, respectively. Based on phytochemical screening, UV and IR spectras and references data, it can be stated that one flavonoid which is apigenin, had been isolated. It showed antiplasmodial activity with percent inhibition of 70.2; 62.5; 39.1; 21.7 and 10.8% at concentrations of 300, 100, 30, 10 and 3 μg/mL, respectively.

**Conclusion:** These findings demonstrated that *Macaranga gigantea* leaves contained flavonoids having potent antiplasmodial property.

**Keywords:** *Macaranga gigantea*, *ex vivo*, *Plasmodium berghei*, antimalaria, flavonoid
In vivo antibacterial activity of *Cassia fistula* L. barks fractions as treatment for resistant pathogenic bacteria

AnisYohana Chaerunisa¹*, Yasmiwar Susilawati², Muhaimin³, and Tiana Milanda²

¹Department of Pharmaceutical Technology, Faculty of Pharmacy, UniversitasPadjadjaran, Sumedang 45363, Indonesia;
²Department of Pharmacy Biology, Faculty of Pharmacy, UniversitasPadjadjaran, Sumedang 45363, Indonesia;
³Department of Chemistry, Faculty of Science and Technology, University of Jambi, Jl. Raya Jambi-MuaraBulian Km 15 Mendalo Indah, Jambi, 36361, Indonesia

*Presenting author
Email: anis.yohana.chaerunisa@unpad.ac.id, Phone: +6281224828105

ABSTRACT

**Background:** Medicinal plants are traditionally used for the treatment of human infections. The increasing incidence of antibiotic resistance necessitates medicinal plants as an alternative for the treatment of resistant infectious organisms. Among resistant bacteria, *Salmonella thyposa* and *Shigella dysenteriae* was used as sample of the study.

**Objective:** To evaluate the antibacterial activity of *Cassia fistula* L. barks fractions as alternative treatment for resistant pathogenic bacteria infections.

**Methods:** Extraction was conducted by maceration using ethanol, followed by partition successively with n-hexane, ethyl acetate and water. *In vitro* antibacterial activities of fractions were tested using microdilution method. The active fraction was examined *in vivo* using an infection mouse model. Mice were initially infected by *Salmonella* – *Shigella* bacteria and then with active fraction of *Cassia fistula* L. Colonies numbers of *Salmonella thyposa* or *Shigella dysenteriae* recovered from Mice’s feces were calculated and concluded antimicrobial activity. Active fraction was subjected to chemical compound analysis through qualitative High Performance Liquid Chromatography (HPLC) profiling.

**Results:** The most active fraction was ethyl acetate with Minimum Inhibitory Concentration (MIC) value as much as 0.625%, followed by water (5%). n-Hexane fraction displayed no visible growth and concluded as inactive fraction. The ethyl acetate fraction was found to have effects on the colonies numbers of *Salmonella thyposa* or *Shigella dysenteriae* from feces samples on *Salmonella* – *Shigella* media for 6 days investigation. The profile spectrums of ethyl acetate fraction from HPLC showed a few peaks that represent the chemicals at the retention time between 7 to 22 minutes.

**Conclusion:** The results of this study indicated that ethyl acetate is the active fraction of *Cassia fistula* L. barks as antibacterial agent and provide potent activity for effective treatment against *Salmonella thyposa* or *Shigella dysenteriae* as samples of resistant bacteria.

**Keywords:** *Cassia fistula* L., resistant bacteria, fractions, *in vivo*, antibacterial agent
Anti-Adipogenic Activity of Fractions of *Guazuma ulmifolia* Leaves Extract

Nuri¹*, Sukardiman², Bambang Prajogo²

¹Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Jember, Indonesia; ²Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Airlangga University, Indonesia.

*Presenting author
Email: nuri.farmasi@unej.ac.id, Phone: 081234904836

ABSTRACT

Objective: The objective of this study was to investigate the anti-adipogenic activity of the chloroform, ethylacetate, and residual ethanol fractions of *Guazuma ulmifolia* leaves extract.

Methods: The fractionation of ethanol extract was carried out by solvent-solvent partition using chloroform and ethyl acetate. Inhibition of fractions to the proliferation and differentiation of primary cultures of rat preadipocytes were tested to investigate anti-adipogenic activity.

Results: Separation of ethanol extract yielded three fractions, i.e. chloroform fraction, ethyl acetate fraction, and residual ethanol fraction. The results of anti-proliferation and anti-differentiation activity test showed that ethyl acetate fraction possessed higher activity than other fractions, followed by residual ethanol fraction and chloroform fraction. The ethylacetate fraction also showed the highest total flavonoid content.

Conclusion: The fraction of ethyl acetate showed the highest anti-adipogenic activity and the highest total flavonoid content.

Keywords: Anti-adipogenic activity, *Guazuma ulmifolia* leaves
Cytotoxic Activity of Selected Medicinal Plants from Papua

Septriyanto Dirgantara¹*, Rosye H.R. Tanjung², Rahmawati Nurlatifah¹ and Edy Meiyanto³

¹Department of Pharmacy, Faculty of Mathematic and Natural Science, Cenderawasih University, Jayapura;
²Department of Biology, Faculty of Mathematic and Natural Science, Cenderawasih University, Jayapura;
³Cancer Chemopreventive Research Center (CCRC), Faculty of Pharmacy, Gadjah Mada University, Yogyakarta

*Presenting author
Email: septriyanto1986@gmail.com, Phone: +6282199491607

ABSTRACT

Background: Some medicinal plants from Papua which have been used traditionally as anticancer agents for many ethnic in Papua Islands. But, scientific evidence for this study were still limited.

Objective: The present study was conducted to test for in vitro Brine Shrimp Lethality Test (BSLT) of the seven selected medicinal plants from Papua, namely, Drymis piperita (local name: Kayu Akway), Myrmecodia beccarii (Sarang semut), Biophytum petersianum (Rumput kebar), Vernonia amygdalina (Daun Afrika), Villebrunea rubescens (Daun Jilat), Laportea aestuans (Daun Gatal) and Breynia cernua (Katuk hutan).

Methods: Seven ethanolic extracts were evaluated for their cytotoxic activity against Artemia salina Leach with concentration extracts from 10;100; and 1000 μg/mL. Cytotoxicity was evaluated in terms of LC₅₀ value from the 24-hour counts using the probit analysis method.

Results: Ethanolic extracts of Myrmecodia beccarii showed the highest cytotoxicity with LC₅₀ was 8.33μg/mL against brine shrimp, but all selected medicinal plants showed the active potential cytotoxic activity because the LC₅₀ value were <1000 μg/mL.

Conclusion: From this research, M. beccarii indicated the possible potential use of medicinal plants from Papua as anticancer agents.

Keywords: Cytotoxic, BSLT, Medicinal Plants, Papua.
Identification of Herbal Mixture Metabolites and Its Acute Toxicity in Wistar Rats

Azliana Abu Bakar Sajak1*, Azrina Azlan1, Hazilawati Hamzah2 and Faridah Abas3

1Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia;
2Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia;
3Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Presenting author
Email: azleeyana@yahoo.com, Phone: +60164226256

ABSTRACT

Background: Herbal mixture (PRM) consist of lemon, ginger, garlic, apple cider and honey has been hailed as one of the remedy in improving and maintaining health condition. However, there is still lack of scientific evaluations on regard to their toxicity as a mixture.

Objective: This study aims to provide the reference frame for the safe dose design of PRM as well as their toxic and adverse effects in the animals for the future efficacy study.

Methods: Identification of metabolites that present in PRM was determined using 1H-NMR measurement with additional support of twodimensional (2D) NMR-J-resolved. The acute toxicity of the PRM was evaluated in Wistar rats. Twelve rats were divided into two groups (n=6 per group, 3 males, 3 females), which are control (C, n = 6) and treated (T, n=6). The T group was given PRM sample (2000 mg/kg bw) dissolved in 1 ml filtered water, while the C group was given filtered water (1 ml). Animals were observed individually after initial dosing for a total of 14 days. Serum samples were collected at the end of the study for biochemical profile.

Results: Identification of metabolites in PRM showed that it contains metabolites such as fructose furanose, lactic acid, ascorbic acid, acetic acid, cycloalliin, pyruvate, proplene-glycol, 5-hydroxymethylfurane, α- and β-glucose. No lethality was observed in the group treated with PRM at the end of the study (14 days). No changes were also observed from the behavioral and appetite of the treated group. No significant changes (p> 0.05) were observed in the biochemical parameters (AST, ALT, total cholesterol, triglyceride, LDL, HDL and creatinine) between the C and PRM groups.

Conclusion: No adverse effects were observed in the group treated with PRM. Therefore, the safe dose for PRM can be up to 2000 mg/kg bw.

Keywords: Acute toxicity, herbal mixture, Wistar rats.
The Cytotoxic Activity of Fractions from the Ethyl Acetate Extract of Rumput Gong (Eriocaulon cinereum R. Br.) on Hela Cervical Cancer Cell

Widyanur Maya Diahandari*, Pinus Jumaryatno, and Arde Toga Nugraha

Department of Pharmacy, Faculty of Science and Mathematics, Universitas Islam Indonesia, Yogyakarta 55548

*Presenting author
Email: 14613092@students.uii.ac.id, Phone: +628979382060

ABSTRACT

Background: Cervical cancer is an abnormal cell growth that occurs in the cervix. The treatment using anticancer agent may affect the health condition and the productivity of the patients due to the side effects. Therefore, a new therapeutic agent with high effectiveness and low side effects is needed. Eriocaulon cinereum, known as rumput gong in Bangka Belitung is a traditional medicinal plant that has been used empirically for treating uncontrolled cell growth. Our previous study showed that the ethyl acetate extract of E. cinereum has antiproliferation activity against Hela cell with IC₅₀ value of 573.75 μg/ml. Because of the promising activity, a further study to evaluate the activity of the fraction from ethyl acetate extract of E. cinereum against the Hela cell was undertaken.

Objective: The aims of this study was to examine the cytotoxic activity of fraction from ethyl acetate extract of Eriocaulon cinereum against HeLa cells.

Methods: E. cinereum was extracted by ultrasound assisted maceration using n-hexane followed by ethyl acetate. The ethyl acetate extract was then subjected to vacuum liquid chromatography to obtain dichloromethane and ethyl acetate fractions. The cytotoxic activity of the fractions against Hela cell was evaluated by MTT assay method and the absorbance value was measured using ELISA reader at the wavelength of 595 nm. The data were analyzed with PROBIT method from SPSS 16 for Windows®.

Results: The fraction of ethyl acetate and dichloromethane of E. cinereum have cytotoxic activity with IC₅₀ value 249.602 μg/mL and 292.681 μg/mL respectively. Selectivity index of ethyl acetate fraction was 2.72 and dichloromethane fraction was 1.41.

Conclusion: The fractions of ethyl acetate extract of E. cinereum showed cytotoxic activities against Hela cell which warrant for further investigation.

Keywords: Eriocaulon cinereum, HeLa cell, cytotoxic activity, Ultrasound Assisted Extraction, MTT assay.
Formulation and Characterization of Cosmetic Serum Containing Argan Oil as Moisturizing Agent

Sri Budiasih1*, Masyitah Ismail1, Jiyauddin Khan 1, Mohammed Kaleemullah1, Samer Al-Dhali1, Fadli Asmani1, Eddy Yusuf 2

1School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; 2International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: sribudiasih@msu.edu.my Phone: +603-55216488

ABSTRACT

Background: Nowadays, cosmetics are becoming more importance in daily life when it was used regularly by number of people in large quantities consumed per year. Moisturizing serum is bland of oleaginous substances that are applied to the skin by rubbing which used to replace natural skin oil, to cover tiny fissures in the skin and to provide a soothing protective film. Argan oil is a plant oil that produced from Argan tree (Argania spinose) which become one of the main roles in the dermocosmetic field due to higher in moisturizer contents.

Objectives: The main objective of the research is to evaluate the characteristics of cosmetic serum and to identify the best formulation of cosmetic serum containing Argan oil as moisturizing agent.

Methods: Five formulations of serum (F1, F2, F3, F4 and F5) with different concentration of Argan oil have been developed. They were evaluated for its physical appearance, pH, rheology, spread ability, skin moisturizing and stability by using ANOVA single factor.

Results: The product was milky white, rose cheek smell, non-greasy, non-oily and homogen. The result showed that pH of five formulations have shown significant different (p<0.05) and was fall into acceptable range (pH 5-6). Stability study at low and room temperature shown all formulation were stable except for high temperature (40°C) only F2 after week 1. Statistical result indicated that there was significant different between all formulations in moisturizing effect (p<0.05) and F3 gave higher percentage of moisture rising. Rheological analysis shows all five formulations were pass over 30% of torque point. Formulation 2, 3 and 4 give more than 50% of spread ability percentage compared with formulation 1 and 5.

Conclusion: In this study, it can be concluded that the F2 of formulated cosmetic serum are stable and could delivered high moisturizer effect on the skin.

Keywords: Cosmetic Serum, Argan oil, moisturizer, stability
Comparison of antioxidant properties of fresh and frozen peel of lemon (*Citrus limon*), key lime (*Citrus aurantiifolia*), and musk lime (*Citrus microcarpa*)

Nur Fatin Inazlina Noor Azman¹, Azrina Azlan¹,²*, Azliana Abu Bakar Sajak¹, and Noor Atiqah Aizan Abdul Kadir¹

¹Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia; ²Research Centre for Excellence for Nutrition and Noncommunicable Disease, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia

*Presenting author
Email: azrinaaz@upm.edu.my Phone: +60 3 8947 2466

ABSTRACT

**Background:** Citrus is known for its high phenolic content and dietary fiber. Unfortunately, the citrus peel is often discarded as waste. Interestingly, fruit waste still contains large amount of bioactive compound. There is a scientific need to initiate an attempt to utilize citrus peel for medicinal purposes.

**Objective:** This study aim to investigate the antioxidant content and activity of fresh and frozen peel of selected citrus.

**Methods:** Frozen and fresh peels of lemon, key lime, and musk lime extracts were screened for their total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties by DPPH free radical scavenging activity and Ferric ion reducing antioxidant power (FRAP) assays.

**Results:** Frozen peel of citrus extracts showed significantly higher TPC and TFC values when compared with fresh citrus peel extracts (p<0.05). Furthermore, frozen citrus peel extracts also showed promising antioxidant activities as indicated by the significantly higher FRAP value when compared with the fresh peel extracts (p<0.05). Moreover, frozen citrus peel also possesses a higher antioxidant activity as indicated by the lower EC₅₀ value which range from 0.823±0.1 mg/ml to 3.16±0.92 mg/ml. in addition, a strong correlation was found between FRAP and TPC (r=0.783), and TFC (r=0.681).

**Conclusion:** Findings of this study suggested frozen peels of citrus can be considered as a reliable source of antioxidant and may potentially be used for various medicinal purposes.

**Keywords:** citrus peel, key lime, musk lime, lemon, antioxidant,
Antibacterial Activities of Ethanol Extract of Karamunting (Melastoma malabathricum L.) Leaf and Flowers on Bacteria Salmonella typhi, Escherichia coli, Staphylococcus aureus

Isnaini1*, Lia Y. Budiarti2, Noor Muthmainah2, Dimas S. Baringgo3, Ririn Frisilia3, Nanda Sulistyaningrum3, Irawati F. Batubara3, Wuri Sofiratmi3, Wiresa D. Renalta3

1Department of Pharmacology and Therapy Faculty of Medicine, University of Lambung Mangkurat Banjarmasin, South Kalimantan, Indonesia; 2Department Mikrobiology Faculty of Medicine, University of Lambung Mangkurat Banjarmasin, South Kalimantan, Indonesia; 3Faculty of Medicine, University of Lambung Mangkurat Banjarmasin, South Kalimantan, Indonesia

*Presenting author
Email: isna_yusuf@yahoo.co.id, Phone: 085248715366

ABSTRACT

Karamunting (Melastoma malabathricum Linn) is native plant of Borneo. This plant has not been used optimally, only considered a pest. M. Malabathricum L contains flavonoid, saponin, tanin and alkoloid. They are having activities of antibacteria. In this study we tested the antibacterial activity of leaf and flower M. malabathricum L against bacteria Salmonella typhi ATCC 14028, Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923. Testing of antibacterial activity using diffusion method by measuring the inhibition zone formed around the paper disk. The results of phytochemical scheme of ethanol extract of M. malabathricum L leaves contain flavonoid, saponin, tanin and alkoloid while ethanolic extract of M. malabathricum Linn flower contain flavonoid, saponin and tanin. The results showed that leaves of ethanolic extract of M. malabathricum L leaves had the greatest inhibitory effect on Salmonella typhi, whereas M. malabathricum L flower had the greatest activity in Escherichia coli with inhibitory diameter of 28.2 mm and 27.9 mm, respectively.

Keywords: Melastoma malabathricum L, Karamunting, Salmonella typhi, Escherichia coli, Staphylococcus aureus
Determination of Standard Specific Parameters Extracts and Activity of Purified Extract Belimbing Wuluh Leaves (Averrhoa bilimbi L.) against Staphylococcus aureus Bacteria

Dyah Aryantini¹*, Luwis Rusiana¹,², and Erma Maszuin³

¹Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Jl. KH. Wachid Hasyim No. 65, Kediri, Indonesia

*Presenting author
Email: dyah.aryantini@iik.ac.id, phone: +6281252788890

ABSTRACT

Background: Belimbing wuluh leaves has great potential as traditional medicines, such as inflammatory and antiinfectives. To obtain the extracts as the main raw material traditional medicines and able to guarantee pharmacological activity it is necessary to determine the standard specific parameters.

Objective: To determine specific parameters 80% ethanol extract of Belimbing wuluh leaves and its purified activity against Staphylococcus aureus.

Methods: Leaves extracts of belimbing wuluh obtained by maceration method with 80% ethanol as solvent. Determination of the standard specific parameters include test of organoleptic, soluble compounds in certain solvent and chemical content of extracts (phytochemical screening and chromatogram profile). Viscus extracts purified to remove ballast substance by washing extracts using different solvents that is n-hexane, ethyl acetate and hot water. The purified extracts was tested for its activity as antibacterial by the Kirby Bauer Diffusion method.

Results: Specific parameters determination of extracts 80% ethanol showed green colour, aromatic typical odor with bitter sense. The soluble ethanol content of the compound was 12.43% w/v and the water soluble was 3.27% w/v. The qualitatively test gives positives result on flavonoids, alcaloids, terpenoids, tannins and saponin. The chromatogram profile obtained specific separation using TLC method with 3 different mobile phase and motion polarity. The activity test of purified extracts showed positive result wich is concentration of purified extracts 80% w/v was giving inhibition zone 18 mm.

Conclusion: The ethanol 80% extracts of belimbingwuluh leaves from Malang, Tulungagung and Kediri meet the general requirements based on General Standard Parameters of Medicinal Plants Extracts.

Keywords: extracts standardization, specific parameters, purified extracts, antibacterial.
Cytotoxic Activity of The Ethanol Extract of
\textit{Platycerium coronarium} (J. Koenig ex O.F.Mull) Desv.
in MCF-7 Breast Cancer Cell

Kristy Tri Wardhani*, Pinus Jumaryatno, and Annisa Fithria

\textit{Department of Pharmacy, Islamic University of Indonesia, Jl. Kaliurang Km. 14.5, Sleman, Yogyakarta, Indonesia 55584}

*Presenting author
Email: 14613149@students.uii.ac.id, Phone: +6289650791727

ABSTRACT

\textbf{Background:} Breast cancer is a leading cause of death worldwide. Due to the high prevalence of breast cancer in Indonesia and the number of problems caused by chemotherapy, new therapeutic agent for breast cancer is needed. \textit{Platycerium coronarium} (J.Koenig ex O.F.Müll.) Desv., known as Paku Tanduk Rusa is a common Indonesian plant that traditionally has been used to treat cancer.

\textbf{Objective:} This study aimed to evaluate the cytotoxic activity of the ethanol extracts of spores, fertile leaves, sterile leaves and fibrous roots of \textit{P. coronarium} and to identify the phytochemical content of the active extracts.

\textbf{Methods:} The extracts were obtained by ultrasound-assisted maceration using ethanol 70%. The phytochemical content of the extracts was screened using standard methods of analysis. The cytotoxicity of the extracts against MCF-7 cells was evaluated using MTT assay method and was measured at an absorbance wavelength of 595 nm using microplate reader. The data was analyzed using linear regression and calculated as IC\textsubscript{50} values (concentrations at which cellular effects are inhibited by 50%).

\textbf{Results:} The fibrous roots extract exhibited a cytotoxic activity against MCF-7 with IC\textsubscript{50} value of 427.76 ppm and Selectivity Index value of 1.14. There were no cytotoxic activities showed by the extracts of spores, fertile leaves, and sterile leaves of \textit{P. coronarium}. The phytochemical screening of fibrous roots extract confirmed the presence of alkaloids, phenolic compounds, flavonoids, and saponins.

\textbf{Conclusion:} The ethanol extract of the fibrous roots of \textit{P. coronarium} has a moderate cytotoxicity against MCF-7 cells but less selective against normal cells.

\textbf{Keywords:} Breast cancer, \textit{Platycerium coronarium}, cytotoxic, MCF-7.
Antiproliferative and Apoptotic Induction of N-Hexane Fraction of Picria fel-terrae Lour. Herbs on T47D Cell Line

Denny Satria1,4*, Jansen Silalahi2, Ginda Haro2, Syafruddin Ilyas3

1Department of Pharmaceutical Biology, University of Sumatera Utara
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sumatera Utara
3Department of Biology, FMIPA, University of Sumatera Utara;
4Faculty of Health Sciences and Pharmacy, Universitas Sari Mutara Indonesia

*Presenting author
Email: denny.satria.dennysatria@gmail.com Phone: +6285296458644

ABSTRACT

Background: A recent study reported that breast cancer is leading in the estimated new cancer cases, and the second most common death cause of women suffering from cancer.

Objective: To evaluate cytotoxic, antiproliferative and apoptotic induction activities of n-hexane fraction (nHF) of Picria fel-terrae Lour. herbs.

Methods: Cytotoxic activity of nHF was determined with MTT method, cell cycle and apoptotic analysis were determined with flow cytometry method towards T47D cell line.

Results: Cytotoxic activity from nHF with MTT assay measured as IC50 was 75.87 ± 0.75 µg/mL, nHF at 15 µg/Ml caused accumulation in G2-M (37.47%) and S phase accumulation (19.41%) and increased early (24.25%) and late apoptosis (4.26%).

Conclusions: The results reveal that nHF of Picria fel-terrae Lour. herbs have antiproliferative and apoptotic induction activities. Our further study is to isolate anticancer compounds from Picria fel-terrae Lour. herbs.

Keywords: Antiproliferative, Apoptotic, Picria fel-terrae Lour., herbs, n-hexane.
Cytotoxic Activity of The Root Extract from Helminthostachys zeylanica (Linn) Hook in MCF-7 Breast Cancer Cell

Herlina Ike Setiyawati1*, Pinus Jumaryatno1, and Annisa Fithria1

1Department of Pharmacy, Islamic University of Indonesia, Jl. Kaliurang Km. 14.5, Sleman, Yogyakarta 55584, Indonesia

*Presenting author
Email: 14613172@students.ui.ac.id, Phone: +6282248876265

ABSTRACT

Background: Breast cancer is the most common type of cancer and a leading cause of the death in women worldwide, including in Indonesia. The side effects due to the chemotherapy lead the need to develop a new anticancer agent. The root of Helminthostachys zeylanica (Linn.) Hook, known as tunjuk langit, has been used traditionally in Indonesia for anticancer agent. Previous study reported that the flavonoids from H. zeylanica such as quercetin, ugonin J and ugonin K have anticanceractivity. However, there is no report of the activity of the root of H. zeylanica in breast cancer cells.

Objective: To investigate the cytotoxic activity of the n-hexane, ethyl acetate and ethanol extracts from the root of H. zeylanica in MCF-7 breast cancer cells and to identify the phytochemical constituents of the extracts.

Methods: The cytotoxic activity of the extracts in MCF-7 breast cancer and vero cells were determined by MTT assay method. The absorbance values obtained from the measurement at a wavelength of 595 nm was analyzed by using a linear regression. The IC50 values (concentrations at which cellular effects are inhibited 50%) was calculated by connecting the logarithm values of the sample concentration as X and the percentage of cell death as Y. The phytochemical screening was performed using standard methods of analysis.

Results: The extracts of n-hexane, ethyl acetate and ethanol from the root of H. zeylanica showed cytotoxic activity against MCF-7 breast cancer with IC50 values of 6.586 μg/ml, 167.081 μg/ml and 619.505 μg/ml, respectively. Meanwhile the selectivity index values of n-hexane, ethyl acetate and ethanol extracts were 7.814, 0.877, and 1.024, respectively. The phytochemical analysis indicated the presence of flavonoids in the extracts.

Conclusion: All the extracts of the root of H. zeylanica have cytotoxic activity. The n-hexane extract showed the most potent cytotoxicity on MCF-7 breast cancer and has good selectivity against normal cells. This study suggests that the root of H. zeylanica is promising source as anti-breast cancer agent.

Keywords: Helminthostachys zeylanica, Breast Cancer, MCF-7, MTT Assay, IC50
Identification of Anticancer Substances of Hexane Fraction from *Agaricus blazei* Murill against MCF-7 Cell-line

Misgiati\textsuperscript{1,2}, Sukardiman\textsuperscript{3*}, Aty Widyawaruyanti\textsuperscript{3}

\textsuperscript{1}Graduate Program, Faculty of Pharmacy, Universitas Airlangga, Surabaya; Departement of Pharmacy; 
\textsuperscript{2}Academy Analys Pharmacy and Food Putra Indonesia, Malang Indonesia; 
\textsuperscript{3}Departement of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya

\textsuperscript{*}Presenting author  
Email: Sukardiman@ff.unair.ac.id, Phone: 081334701501

ABSTRACT

Research has been done to find out the anticancer active ingredient of *Agaricus blazei* Murill on MCF-7 cell lines. *Agaricus blazei* Murill contains ergosterol, blazein, agaritin, agariblazepirol C. The method used for fractionation is Bioassay guided fractionation, to identify active substances using FTIR, NMR-\textsuperscript{1}H, NMR-\textsuperscript{13}C, LCMS, methods for determining anticancer activity in cells MCF-7 with MTT method.

**Result:** Results obtained from the hexane fraction process of 19.62 grams, there are three subtractions namely SFH1, SFH2, and SFH3, after identification on the subfraction by using TLC containing the class of terpenoid compounds. Each subfraction weighed for SFH1 0.045 gram with IC\textsubscript{50} 0.03 μg/ml, SFH2 0.5367 gram with IC\textsubscript{50} 0.074 μg/ml, and SFH3 0.6324 gram with IC\textsubscript{50} 0.288 μg/ml. SFH3 subfraction is available two stains recrystallized with methanol solvent. The resulting crystals were performed with TLC showing a stain. The results of H-NMR and C-NMR have not shown pure compounds, nor is it proved by LC-MS results.

**Conclusion:** The results of the terpenoid group compounds in each subfraction, the SFH3 subfraction being not yet pure compounds, the anticancer activity of IC\textsubscript{50} of each 0.03 μg/ml, 0.074 μg/ml, 0.288 μg/ml

**Keywords:** ..................
The Cytotoxic Activity of Fractions from the Methanol Extract of Rumput Gong (Eriocaulon cinereum R. Br.) on MCF-7 Breast Cancer Cell

Denox Asih Pratiwi, Siti Nurul Komariah, and Rusdiana Kartika Sari

1Department of Pharmacy, Faculty of Science and Mathematics, Universitas Islam Indonesia, Yogyakarta 55548, Indonesia

*Presenting author
Email: 15613110@students.ui.ac.id, Phone: +6281331499707

ABSTRACT

Background: In 2013, Kementerian Kesehatan RI mentioned that breast cancer was a cancer with highest prevalence in Indonesia, there was breast cancer up to 0.5‰. The prevalence of breast cancer found in D.I. Yogyakarta at 2.4%. Eriocaulon cinereum R. Br. was a traditional medicinal plant that has been used empirically for treating uncontrolled cell growth by people in Indonesia especially Bangka Belitung.

Objective: The aim of this research is to know the cytotoxic activity of dichlorometane and water fraction from methanol extract of Eriocaulon cinereum against MCF-7 cells and Vero cells.

Methods: The extraction was done through Ultrasound Assisted Extraction. Fractionation was done by using the liquid partition method. The cytotoxic activity of the fractions was evaluated by MTT assay method and the absorbance value was measured using ELISA reader at the wavelength of 595 nm. The data were analyzed with PROBIT method from SPSS 16 for Windows®.

Results: The result of cytotoxic test showed that the value of Inhibition Concentration (IC50) of dichlorometane fraction against MCF-7 cell was 459.856 μg/ml, while IC50 of water fraction was 5473.584 μg/mL. IC50 fraction of dichloromethane against Vero cell is 341.524 μg/mL and IC50 the water fraction is 5296.779 μg/mL. The selectivity index (IS) of the dichloromethane fraction is 0.743.

Conclusion: Based on IC50 values, it can be concluded that the cytotoxic activity of the dichloromethane fraction is weak and the water fraction has no cytotoxic activity against MCF-7 cells and Vero cells. The result of selectivity analysis showed that the gum dichlorometane fraction was less selective on MCF-7 cells.

Keywords: Eriocaulon cinereum, MCF-7 cell, cytotoxic activity, Ultrasound Assisted Extraction, MTT assay.
Cytotoxic evaluation of Fractions from Methanol Extract of Rumput Gong 
(*E. Cinereum, R. Br.*) on Hela Cervical Cancer

Auva Azkiya¹*, Pinus Jumariyatno¹, and Arde Toga Nugraha¹

¹Department of Pharmacy, Faculty of Science and Mathematics, Universitas Islam Indonesia, Yogyakarta 55548, Indonesia

*Presenting author
Email: 14613208@students.uii.ac.id, Phone: +6281248102846

ABSTRACT

Background: *Eriocaulon cinereum* R. Br., known as rumput gong, has been used by the people in Bangka Belitung as traditional medicine to prevent uncontrolled cell growth. Preliminary study reported that the ethanol extract of *E. cinereum* was able to inhibit cell division of Hela cervical cancer with IC₅₀ value of 427.79 μg/ml. This showed that this herb is potential to be developed as anticancer. Therefore, a further study was carried out to evaluate the cytotoxic activity of the fractions from the methanol extract of *E. cinereum* on Hela cell.

Objective: The aim of this study was to examine the cytotoxic activity of fraction extract methanol of Rumput Gong (*Eriocaulon cinereum R. Br.*) against cervical cancer cells (HeLa) and normal cells (Vero).

Methods: The ultrasound assisted maceration was performed to extract the *E. cinereum* using gradient polarity from n-hexane, ethyl acetate and methanol. The methanol extract was subjected to vacuum liquid chromatography to obtain dichloromethane, chloroform and water fractions. The evaluation of cytotoxic activity of the fractions was undertaken by MTT assay method, meanwhile the absorbance value was obtained using ELISA reader with the wavelength of 595 nm. Due to the amount of the chloroform fraction, no activities evaluation was carried out. The data were analyzed using PROBIT method from SPSS 16 for Windows®.

Results: The fraction of dichloromethane from methanol extract of *E. cinereum* showed cytotoxic activity with IC₅₀ value of 235.652 μg/mL. Meanwhile the water fraction has no cytotoxic activity due because its IC₅₀ value was 2325.302 μg/mL, selectivity index value of fraction dichloromethane was 2.09 and water fraction was 2.13.

Conclusion: The dichloromethane fraction from methanol extract of *E. cinereum* has cytotoxic activity against Hela cell.

Keywords: *Eriocaulon cinereum*, HeLa cell, cytotoxic activity, Ultrasound Assisted Extraction, MTT assay.
The ACE inhibitory activity of protein hydrolysates from local sea cucumbers

Ariyanti S. Dewi1*, Gintung Patantis1, Yusro N. Fawzya1, Hari E. Irianto1, Siti Sa’diah2

1Research Center for Marine and Fisheries Product Processing and Biotechnology, Jl. Petamburan VI, Jakarta 10260, Indonesia;
2Tropical Biopharmaca Research Center, IPB,Jl. Taman Kencana 3, Bogor 16128, Indonesia

*Presenting author
Email: ariyantisd@gmail.com, Phone: 085803424959

ABSTRACT

Hypertension is one of the risk factor that contributes significantly to the prevalence of cardiovascular diseases in Indonesia. Medical treatments for hypertension therapy are not only causing a high economic burden to patients, but also diverse side effects. Peptides have been known to exhibit anti hypertension activities. Peptides for the treatment of hypertension in Indonesia are mostly imported and some of them are derived from bovine and porcine that cause concerns for Indonesian consumers due to religious reasons. We are thus targeting marine biota, such as sea cucumbers, as the alternative peptide producers. Among 80 species of sea cucumbers in Indonesia, only 10% of them that have been exploited commercially. The rest are of low or medium values and their utilization has not been fully optimized. We isolated protein hydrolysates containing collagen peptides from three local sea cucumbers (Bohadschia sp., Holothuria sp., Stichopus sp.) using three commercial protease enzymes (alkalase, neutrase, flavourzyme) then tested the extracts for their anti-hypertension activity using the angiotensin-converting enzyme (ACE). We found that protein hydrolysates from local sea cucumbers showed significant activity as ACE inhibitors and the protease enzyme used for the hydrolysis process affected their inhibitory activities.

Keywords: sea cucumbers, collagen peptides, protein hydrolysates, hypertension
Assay of β-carotene from *Spirulina maxima* by Spectrophotometry Visible

Siti Fatmawati Fatimah\(^1\), Vani Aisyah\(^2\), Laela Hayu Nurani\(^2\), Citra Aryani Edityaningrum\(^1\)

\(^1\)Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, 55164;
\(^2\)Department of Biology Pharmacy and Phytochemical, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, 55164

*Presenting author*
Email: fatmafatima28@gmail.com, Phone: +6285659359137

ABSTRACT

**Background**: According to Food Drug Association (FDA), malnutrition can be resolved by Spirulina with a high β-carotene content (10 mg) of spirulina powder (3 gr). A presented method was established for the identification and quantification of β-carotene from *Spirulina maxima* ethanol extract 96% with Spectrophotometry Visible.

**Objective**: Develop analysis method of β-carotene from *Spirulina maxima* ethanol extract 96% with Spectrophotometry Visible.

**Method**: The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, limit of detection (LOD), and limit of quantification (LOQ), with λ 454 nm, and acetone as β-carotene solvent.

**Result**: Analytical methods validation of β-carotene in *Spirulina maxima* ethanol extract 96% qualified for linearity with r= 0.997 (specification: r value ≥ 0.98), RSD of repeatability 1.144% (specification: RSD ≤ 2.0%), RSD of intermediate precision 0.987 (specification: RSD ≤ 2.0%), accuracy with recovery range 98.765%-101.667% (specification: 98.0%-102%), The LOD 0.354 ug/mL and LOQ 1.181 ug/ml.

**Conclusion**: Presented method was established simple, valid, and suitable for routine quantitative and qualitative determination of β-carotene in *Spirulina maxima*.

**Keywords**: *Spirulina maxima*, β-carotene, analytical methods validation.
Evaluation of Antioxidant and Cytotoxic Activities of *Vernonia amygdalina* Del. Leaves

Poppy Anjelisa Zaitun Hasibuan¹*, Urip Harahap¹, Panal Sitorus², Denny Satria²

¹Department of Pharmacology, University of Sumatera Utara, Medan
²Department of Pharmaceutical Biology Faculty of Pharmacy, University of Sumatera Utara, Medan 20155, Indonesia

*Presenting author
Email: poppyanjelisa@usu.ac.id, Phone: +6281260163104

ABSTRACT

**Background:** The excessive production of oxygen free radicals and the unbalanced mechanism of antioxidant protection results in the onset of many diseases such as breast cancer.

**Objective:** To evaluate antioxidant and cytotoxic activities of n-hexane, ethylacetate and ethanol fraction of *Vernonia amygdalina* Del. leaves.

**Methods:** Antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazil (DPPH) method and cytotoxic activity was determined with MTT method towards T47D cell line.

**Results:** Antioxidant activity from n-hexane, ethylacetate, ethanol fractions and quercetine as positive control with DPPH assay measured as IC₅₀ were 297.33 ± 0.46; 177.99 ± 0.32; 37.92 ± 1.03 and 2.32 ± 0.01 µg/mL, respectively. Cytotoxic activity from n-hexane, ethylacetate, ethanol fractions and doxorubicin as positive control with MTT assay measured as IC₅₀ were 327.89 ± 1.13; 64.92 ± 0.72; 1591.75 ± 37.05 and 1.82 ± 0.05 µg/mL, respectively.

**Conclusions:** The results reveal that fractions of *Vernonia amygdalina* Del. leaves have antioxidant and cytotoxic activities. Our further study is to asses anticancer mechanism of *Vernonia amygdalina* Del. leaves.

**Keywords:** Antioxidant, Cytotoxic, *Vernonia amygdalina* Del., leaves, fraction.
Synthesis of 4-Hydroxycinnamic Acid from Malonic Acid and 4-Hydroxybenzaldehyde as Starting Material with Variation of Catalysts

Iin Narwanti* and Dewi Apriyanti

Faculty of Pharmacy, Universitas Ahmad Dahlan, Jl. Prof. Dr. Soepomo Janturan Yogyakarta, Indonesia

*Presenting author
Email: iin.narw@gmail.com, Phone: 082220183993

ABSTRACT

The purpose of this study was to synthesize 4-hydroxycinnamic with variation of the catalysts through Knoevenagel condensation reaction to obtain optimum rendemen. Synthesis of 4-hydroxycinnamic uses malonic acid and 4-hydroxybenzaldehyde as starting materials. Catalysts that used in this study are pyridine, piperidine, and pyridine-piperidine (2:1). Reflux process lasted for 5 hours, at a temperature of ± 80°C. Thus, the compound was isolated and recrystallized, then the rendemen is calculated. The compound’s purity was identified based on melting point data, TLC and gas chromatography. UV-Vis spectrophotometry, IR spectrometry, Mass spectrometry, and 1H-NMR were used to identify structure compounds. The results showed that the average rendemen obtained on the variation of the catalyst is pyridine-piperidine, piperidine, and pyridine consecutively (82.45±5.75)%; (59.62±4.73)% and (36.51±3.03)%, with pyridine-piperidine catalyst group resulting optimal rendemen. The results of TLC and gas chromatography showed that the product is a pure compound. Based on the identification by UV-Vis spectrophotometry, IR spectrophotometry, mass spectrometry and 1H-NMR, that compound synthesized is 4-hydroxycinnamic acid.

Keywords: synthesis, 4- hydroxycinnamic acid, Knoevenagel condensation reaction, catalyst variation
Cell Cycle Arrest Activity of Alkaloid Fraction of *Litsea cubeba* Lour. Heartwoods Towards HeLa Cancer Cell

Aminah Dalimunthe\(^1\)\(^*\), Poppy Anjelisa Zaitun Hasibuan\(^1\), Denny Satria\(^2\)

\(^1\)Department of Pharmacology, University of Sumatera Utara, Medan
\(^2\)Department of Pharmaceutical Biology Faculty of Pharmacy, University of Sumatera Utara, Medan

\(20155,\) Indonesia

\(*\)Presenting author

Email: aminahdalimunthe@usu.ac.id, Phone: +6281260773719

ABSTRACT

**Background:** Cervical cancer treatment with chemotherapeutic agents is limited because of drug resistance problem and toxic effect on normal tissue leads to immunosuppression and cardiotoxicity.

**Objective:** This study was to investigated cell cycle arrest activity towards HeLa cell lines of *Litsea cubeba* Lour. heartwood alkaloid fraction.

**Methods:** *Litsea cubeba* Lour. heartwood powder was extracted by maceration method with ethanol 96% and fractionated with n-hexane and chloroform at pH 3.7 and 9. Cytotoxic study was using MTT method and analysis cell cycle was using flow cytometry method.

**Results:** The IC\(_{50}\) of ethanol extract, n-hexane and chloroform fractions at pH 3.7 and 9 at were 156.24 ± 2.96; 67.23 ± 0.63; 175.92 ± 2.40; 52.46 ± 0.34; and 94.81 ± 2.16 µg/mL respectively. The chloroform fractions at pH 7 concentration 25 and 10 µg/mL were caused accumulation in G\(_2\)-M phase (33.84 and 29.08%).

**Conclusions:** The results reveal that *Litsea cubeba* Lour. Heartwood alkaloid fraction provide effective as cell cycle arrest. Our further study is to assess the mechanism of alkaloid fraction in inhibit metastasis in cervic cancer.

**Keywords:** Cell cycle, *Litsea cubeba* Lour. heartwood, alkaloid fractions, HeLa.
The Antibacterial Activity of Isolated Flavanoid Fractions from Ethanolic Peel of Citrus sinensis (Valencia Orange) with Citrus Limon (Lemon) against Staphylococcus aureus and Pseudomonas aeruginosa

Nik Nur Shamiha, Nik Dzulkefli¹*, Ghayatery Nagatamby¹, Jiyauddin Khan¹, Fadli Asmani¹, Eddy Yusof²

¹School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; ²International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: eddy@msu.edu.my, Phone: +603-55216488

ABSTRACT

Background: Skin and soft tissue infections (SSTIs) are most common infections encountered by all physicians. Even though pharmacological industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased.

Objective: The aim of this study is to evaluate the antibacterial activity of isolated flavonoid fractions from ethanolic peel extract of C. sinensis (Orange), C. limon (Lemon) and its combination against S. aureus and P. aeruginosa.

Methods: The ethanolic peel extract of both plant were screened for phytochemical identification of flavonoid by lead acetate test and shinoda test. The extract of both plants were evaluated for preliminary antibacterial activity using disk diffusion method. Thin-layer chromatography and column chromatography was performed to isolate flavonoids. Isolated flavonoids were subjected to determination of minimum inhibitory concentration and antibacterial assay by disk diffusion method.

Results: Isolated flavonoids of C. sinensis (17 mm, 8 mm), C. limon (20 mm, 9 mm) and its combination (24 mm, 14 mm) produced antibacterial activity that is comparable to the Ciprofloxacin disc (30 mm, 9 mm) against S. aureus and P. aeruginosa respectively. Thus, these results suggested that C. limon produced a better antibacterial activity against both bacteria compared to C. sinensis. However, the combination of both plants isolated flavonoid fractions produced much better antibacterial activity against S. aureus and P. aeruginosa in comparison with individual flavonoid fractions of both plants.

Conclusion: Therefore, Citrus fruits peels that is being as primary waste in juicing industries can be further developed as marketable natural source of antibiotic as a treatment of SSTIs.

Keywords: Citrus sinensis; Citrus limon; Combination antibacterial activity; Flavonoid; Skin and soft tissue infections (SSTIs)
Mechanism of Antimicrobial Action of *Ocimum Basilicum* Essential Oil against Nosocomial Bacteria

Fadli Asmani¹*, Kiran Chanabasappa Nilugal¹, Sherilyn Fenn Karel¹, Santosh Fattepur¹, May Florence Dela Cruz Bacayo¹, Wong Chau Ng Choon¹, Rasny M¹ and Eddy Yusuf ²

¹School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; ²International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: m_fadli@msu.edu.my Phone: +603-55216488

ABSTRACT

**Background:** Nosocomial infection is a major concern in the healthcare sector. *Ocimum basilicum* essential oil is one of the main essential oil being investigated for its antimicrobial activities.

**Objectives:** This study is conducted to determine the effect of *Ocimum basilicum* essential oil on bacterial cell growth and cell membrane integrity.

**Method:** The *Ocimum basilicum* essential oil (EO) was purchased at Natur Aromatherapy and Wellness and different concentrations of the essential oil (0.092 – 1.470 g/ml) was prepared by diluting it with 10% DMSO. The bacteria used were *Escherichia coli* and *Staphylococcus aureus* while the positive control was Gentamicin antibiotic. Disc diffusion test and MIC using broth dilution was conducted. Growth kinetic analysis of the bacteria was done for a period of 24 hours. Bacterial cell membrane integrity was investigated by measuring the absorbance value using UV Spectrophotometer.

**Result:** The zone of inhibition by the essential oil was greater in *S.aureus* compared to *E.coli* and the MIC is 0.368 g/ml in *S.aureus* and 0.735 g/ml in *E.coli*. EO concentrations at 1.47 g/ml and 0.735 g/ml are the only one showed effective inhibition on the growth of the bacteria. The EO was more effective in inhibiting the growth of *S. aureus* than *E.coli*. It was found that the absorbance of the bacterial cell constituents increased from the negative control, MIC, 2x MIC and Gentamicin in both bacteria.

**Conclusion:** *Ocimum basilicum* essential oil antimicrobial mechanism of action is by acting on cell membrane integrity of the bacteria which caused inhibition of the bacteria growth.

**Keywords:** Essential oil, *Ocimum basilicum*, nosocomial infection, Antimicrobial mechanism
Optimization of Klabet (Trinonella Foenum-Graecum) Dry Extract using Response Surface Methodology

Damai Ria Setyawati*, Lestari Pudjiastuti

Pusat Teknologi Farmasi dan Medika, Badan Pengkajian dan Penerapan Teknologi (BPPT)

*Presenting Author
Email: damai.ria@bppt.go.id, Phone: 0217560707

ABSTRACT

Klabet possess antidiabetic, hypocholesterolemia, antilipidemia, antioxidants and antiinflammatory activity. Formula of dry extract was obtained through optimization of response surface methodology to get optimal physicochemical properties of dry extract. Optimization is done using Minitab software version 14. The initial optimization of drying extract obtained the selected fillers are amprotab and MCC PH102. It was found that dry extract formulas have poor flow property, so it is necessary to add adsorbent. The chosen adsorbent to improve the flow property of dry extract was 1% aerosil. Based on the result of response optimization, the optimal formula was extract: MCC: amprotab 1: 1.3244: 0.7929, response of flow time of 3.5 seconds; water content 4.75%; loss on drying 9.13% and total phenolic content 0.67%. The characterization results of dry extract optimal formula close to the expected target so that it can be used to optimize the manufacture of dry extract.

Keywords:
Mechanism of actions of green tea epigallocatechin-3-gallate (EGCG) targeting iron chelation activity and endoplasmic reticulum (ER) stress pathway in colorectal cancer cells

Zarith Nameyrra Md Nesran, Nurul Husna Shafie*, Norhaizan Mohd Esa, and Amin Ismail

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Presenting author
Email: nhusnashafie@upm.edu.my, Phone: +60389472470

ABSTRACT

**Background:** Green tea has long been known as a timeless healthy beverage and the application of green tea has now been extended to food and pharmaceutical products. One of the most abundant bioactive compounds in green tea, epigallocatechin-3-gallate (EGCG) has demonstrated its role as the key player in various diseases treatment and prevention including colorectal cancer.

**Objective:** This study aims to combat the colorectal cancer incidences by investigating the EGCG’s potential as an iron chelator and its mechanism in inducing endoplasmic reticulum (ER) stress in colorectal cancer cells, HT-29.

**Methods:** The HT-29 cells were initially treated with 88µM (IC₅₀ value) of EGCG for 24h, 48h and 72h. The expression of iron-regulated and ER stress sensor proteins were determined using Western blotting.

**Results:** The expression of iron-regulated proteins; transferrin receptor (TfR) was up-regulated and ferritin-H (FtH) was down-regulated indicating the iron chelation activity by EGCG. The ER stress key proteins, Inositol-Requiring Enzyme 1 α (IRE1α) and Pancreatic ER Kinase (PKR)-like ER kinase (PERK) and as well as its downstream targets; eIF2α, p-eIF2α and ATF4 were up-regulated after incubated with EGCG in HT-29 cells.

**Conclusion:** Green tea EGCG has demonstrated its potential as an iron chelator and ER stress inducer in colorectal cancer cells. The ER stress induction by EGCG can potentially further induce cells apoptosis and therefore, green tea EGCG could be a pharmacological agent in combating colorectal cancer.

**Keywords:** Catechins, iron chelation, endoplasmic reticulum, colon cancer
Comparison of Two Different Immunochromatography Rotavirus Kit for accurate and reliable detection in stool specimens of pediatric patients in Indonesia

Zayyin Dinana¹, Rury Mega Wahyuni¹, Laura Navika Yamani¹,⁵, Juniastuti¹,², Soegeng Soegijanto¹,⁴, Ishak Samuel Wuwuti¹, Maria Inge Lusida¹,², Soetjipto¹,³

¹Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia; ²Departement of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya 60115, Indonesia; ³Departement of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya 60115, Indonesia; ⁴Departement of Pediatric, Faculty of Medicine, Universitas Airlangga, Surabaya 60115, Indonesia; ⁵Departement of Epidemiology, Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia

*Presenting Author
Email: dzayyin@gmail.com, Phone: 085235721712

Abstract

Backgrounds: The incidence of diarrhea caused by diarrhea in Indonesia was occurred during throughout the year. Deaths of children in Indonesia reach about 240,000 per year and the highest cases under 5 years old of age. However, few data is available about diagnose of rotavirus causing gastroenteritis especially in pediatric patients in hospital. Nowadays immunochromatography rotavirus kit is commonly used as a fast and easy method to diagnose of rotavirus.

Objectives: The purpose of this study to compared of two different immunochromatography rotavirus kit for detection of rotavirus eventually confirmed by Reverse Transcription Polymerase Chain Reaction (RT-PCR) test.

Methods: Twenty stool samples were collected from hospitalized children patients (age range: 3 months - 3 years) with diarrhea from the hospital in Indonesia start from April 2017 to December 2017. Our study used One step Rotavirus antigen test Dipstick ‘Eiken’ Rota (Dipstick) and SD BIOLINE Rotavirus for diagnostic infection from stool samples.

Result: An investigation using 20 stool samples showed that the relative sensitivity and specificity compared with a gold standard RT-PCR that Dipstick ‘Eiken’ Rota (Dipstick) were 100% and 81.8%, while SD BIOLINE Rotavirus were 55.55% and 100%, respectively. Based on this result, Dipstick ‘Eiken’ Rota (Dipstick) was more sensitive compared with SD BIOLINE Rotavirus.

Conclusion: Dipstick ‘Eiken’ Rota (Dipstick) would be useful as routine test in hospital in Indonesia because more sensitive compared with SD BIOLINE Rotavirus also its rapid and ease of practice for the diagnosis of rotavirus infections in children.

Keywords: Rotavirus, Immunochromatography Rotavirus Kit, RT-PCR
Antimalarial Active Prenylated Flavonoid from Artocarpusaltilis Leaves Extract

Agriana Rosmalina Hidayati, Hilkatul Ilmi, Aty Widyawaruwanti, Din Syafruddin, and Achmad Fuad Hafid

1Department of Pharmacology, Faculty of Medicine, Universitas Mataram, Mataram 83125, Indonesia
2Post Graduate Student of Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60826, Indonesia
3Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60826, Indonesia
4Natural Product Medicine Research and Development Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia
5Eijkman Institute for Molecular Biology, Jakarta, Indonesia.

*Presenting Author
Email: agriana.rh@gmail.com; Phone: 6281252339933

ABSTRACT

Background: Artocarpusaltilis (known as sukn in Indonesia) leaves extract was reported as a potential candidate of antimalarial drug which exhibited antimalarial activity in vitro against Plasmodiumfalciparum and in vivo against P. berghei with inhibition concentration (IC50) and effective dose value (ED50) of 1.32µg/ml and 0.82mg/kg, respectively. The antimalarial active compound from this plant was not identified yet.

Objective: to identify the active compound from ethanol extract of Artocarpusaltilis leaves and their antimalarial activities

Methods: A. altilis leaves extract was separated by VLC method. All fractions from A. altilis leaves extract were tested for antimalarial activities. In vitro antimalarial activity test was conducted using P. falciparum (3D7 strain) culture in RPMI-1640 medium. The sample prepared in serial dilution at 5 concentrations. After 48 hours incubation with parasite thin blood smears were made, the percentage of parasitaemia was determined by counting infected red blood cells. The most active antimalarial fraction was then isolated with high performed liquid chromatography preparative. The isolate from active fraction was identified by High Performance Liquid Chromatography (HPLC). The pure compounds obtained in the present investigation were subjected to HPLC, 1D and 2D (1)H-Nuclear magnetic resonance (NMR), (13)C NMR, and COSY.

Results: Extract was separated into six fractions and AAL-V4 showed yellow spot intensively and was the most active fraction with IC50 0.03 µg/ml. Purification of AAL-V4 led to the isolation of 3 isolate, AAL-I1, AAL-I2, and AAL-I3. The pure compounds, AAL-I2 obtained in the present investigation were subjected to 1D and 2D (1)H-Nuclear magnetic resonance (NMR), (13)C NMR, HMBC, COSY and HMQC. Based on the spectral analysis and compared to references study, it is concluded that the isolated compounds were prenylated flavonoid compound with IC50 value against P. falciparum1.45 µM.

Conclusion: AAL-I2 was showed active substance against P. falciparum, and was classified as very active against P. falciparum with IC50 value of 1.45 µM. It was identified as a prenylated flavonoid compound and might be a potential candidate of the antimalarial drug from Artocarpus species.

Keywords: Artocarpusaltilis, flavonoid, antimalarial activity, Plasmodiumfalciparum
Amoebicidal activities of Indonesian medicinal plants in the Balikpapan East Kalimantan

Fendi Yoga Wardana¹*, Defi Kartika Sari², Myrna Adianti², Adita Ayu Permanasari², Lidya Tumewu², Aty Widyawaruyanti¹,² and Achmad Fuad Hafid¹,²

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia;
²Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia

*Presenting author
Email: fendi.w7@gmail.com, Phone: +6285755171947

ABSTRACT

Background: Entamoeba histolytica is protozoan agent causing human amoebiasis and responsible for 100,000 deaths annually throughout the world. Recommended in the treatment of amoebiasis using metronidazole has been reported to be less effective, because of the drug resistance effect by Entamoeba histolytica. Therefore, the search of new drugs with amoebicidal activity is important. The natural substances from medicinal plants are potentially a good target to be studied.

Objective: To evaluated Indonesian medicinal plants for their anti-amoebic activities.

Methods: The hexane, dichloromethane and methanol extracts of 114 samples derived from 22 species of medicinal plants explored in the Balikpapan forest, East Kalimantan has been tested. Their anti-amoebic activity was determined by in vitro cell-based assay against Entamoeba histolytica strain HM-1:IMSS (clone 6).

Results: According to cell-based assay, five of 114 samples tested showed anti-amoebic activities. The highest anti-amoebic activity was obtained from the dichloromethane extract of Cratoxylum sumatranum stem bark with 50% inhibitory concentration (IC₅₀) of 22.07 ± 0.84 μg/ml. Subsequently, the dichloromethane extract of leaves and the dichloromethane extract of stem from Garcinia parviflora with IC₅₀ of 38.59 ± 9.46 μg/ml and 68.34 ± 0.4 μg/ml, respectively. The hexane extract of stem bark and the dichloromethane extract of stem from Cratoxylum sumatranum with IC₅₀ of 69.79 ± 16.58 μg/ml and 118.49 ± 15.26 μg/ml, respectively.

Conclusions: The dichloromethane extracts of Cratoxylum sumatranum stem bark and Garcinia parviflora leaves are the most potential candidates in the development of anti-amoebic drugs.

Keywords: Amoebiasis, anti-amoebic, Indonesian medicinal plants, Entamoeba histolytica.
Effect of soaking and boiling on the proximate composition, total phenolic and flavonoid content of Chia seed (*Salvia hispanica* L.) and Flaxseed (*Linum usitatissimum* L.)

Ong Li Ting and Siti Raihanah Shafie*

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Presenting author

Email: sitiraihanah@upm.edu.my, Phone: +60386092973

ABSTRACT

**Background:** Chia seed and flaxseed are popular as functional foods due to their nutritional composition and health-promoting properties. Processing methods have been reported to improve these properties of plant seeds. However, there are limited studies being done on the effects of soaking and boiling on the nutritional and antioxidant compositions of chia and flax seed.

**Objective:** The aim of this study is to investigate the effect of soaking and boiling on the proximate composition, total phenolic content and total flavonoid content of chia seed and flax seed.

**Methods:** Black Chia seed and golden flax seed were soaked in distilled water (25 ± 3 °C) overnight for 24 hours and boiled in distilled water at 95 ± 3 °C for 15 min. The proximate composition (moisture, ash, carbohydrate, protein and fat), total phenolic and flavonoid content of raw, soaked, and boiled chia and flax seed were determined in this study.

**Results:** The moisture content of chia seeds increased after soaking (10.39 ± 0.02%) and boiling (17.46 ± 0.11%). Meanwhile, the moisture content of flax seed (7.01 ± 0.00%) decreased after soaking (6.28 ± 0.00%) and boiling (5.69 ± 0.12%). There was no significant difference between raw chia, soaked chia, and boiled chia, as well as between raw flax, soaked flax, and boiled flax (p > 0.05) in terms of ash content. The protein content was highest in raw chia seed (17.68 ± 0.03/100g) and it decreased after being boiled (15.90 ± 0.30/100g) and soaked (14.44 ± 0.11/100g). In contrasts, the protein content of flax seed (15.13 ± 0.11/100g) increased after being boiled (16.35 ± 0.34/100g) and soaked (16.46 ± 0.04/100g). In this study, soaking and boiling could not change the fat content of chia seeds. The fat content of flax seed (32.84 ± 0.80%) was found to be higher than chia seed (29.33 ± 0.51%). Boiling of chia and flax seed were found to be increased in the total carbohydrate content. Total phenolic content for both chia and flax seed were found to be increased after being soaked and there was no effect on soaking of both chia seed and flax seed on total flavonoid content.

**Conclusion:** Soaking and boiling of chia seed and flax seed could maximize or reduce the proximate composition and antioxidant properties of both seeds.

**Keywords:** Chia seed, flax seed, proximate, total phenolic, total flavonoid
Quality Standardization of Dry Powder of *Spilanthes acmella* Murr. Herb

Ridho Tryantono*, Neny Purwitasari and Retno Widyowati

*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya 60285*

*Presenting author*
Email: ridhotryantoro@gmail.com, Phone: +6285718774943

**ABSTRACT**

**Background:** *Spilanthes acmella* Murr. is a plant that has various medical values, one of them is to overcome osteoporosis. This plant is very potential to be developed into traditional medicine products. Based on this purpose, the raw materials as crude drug and extract should have been standardized. But the standard parameter value of this plant is not yet available on Indonesia Herbal Pharmacopoeia. It is therefore necessary to set the standard parameter values of crude drug and extract of *Spilanthes acmella* Murr.

**Objective:** The aim of this study is to determine the standard parameter value of crude drug from *Spilanthes acmella* Murr. leaves which grow in Purwodadi, East Java, Indonesia.

**Methods:** The method used in this study is in accordance to the requirement of Indonesian Herbal Pharmacopoeia. The total steroid content of this plant was determined using colorimetric method.

**Results:** The results showed that the water-soluble concentration, ethanol-soluble concentration, the total value of as hand the total value of acid insoluble of crude drug were 15.62%, 3.27%, 13.41% and 2.55%, respectively.

**Conclusion:** This research can provide information for standardization of plant material.

**Keywords:** *Spilanthes acmella*, dry powder, standardization.
The Effectivity of Butanol Fraction of Calophyllum nodosum as Antiviral Drug to Dengue Virus Serotype 2

Syifa Salsabila¹, Nabilla Calista¹, Hidayati Desti², Beti Ernawati Dewi²

¹Undergraduate Student, Faculty of Medicine, Universitas Indonesia;
²Department of Microbiology, Faculty of Medicine Universitas Indonesia- Cipto Mangunkusumo Hospital

*Presenting Author
Email: nabilla.calista@gmail.com, Phone: 08118893930

Abstract

Introduction: Dengue fever still high incidence rate in the world including in Indonesia. Until now, there is no specific antiviral drug for DENV infection. Research development for DENV antiviral drug from herbal sources had been done. Calophyllum nodosum which as known to have antimicrobial activity may have potential as antiviral.

Objectives: The objective of this study was to evaluate the antiviral effects of butanol fraction of Calophyllum nodosum on DENV-2 activity in vitro.

Method: In this study, we used DENV serotype 2 New Guenea strain and Huh7it-1 human hepatoma cell line. Beside IC₅₀ value, we also concern on cytotoxic effect on the cell (CC₅₀ values). The IC₅₀ value were determined by focus assay using various concentrations extract of 80, 40, 20, 10, 5, and 2.5 μg/mL, respectively. The CC₅₀ value showed the effect of cytotoxic extract and resulted from MTT assay using concentrations of 640, 320, 160, 80, 40, 20, and 10 μg/mL. Selectivity index (SI) was determined by ratio of CC₅₀ and IC₅₀.

Results: The infective DENV were not found after treated with concentration more than 40 μg/mL. At the highest concentration of toxicity assay, the viability of the cell still same with control DMSO. Statistical analysis showed significant differences between control group and treatment group on results of focus assay and MTT assay. The IC₅₀, CC₅₀ and SI value of butanol fraction of Calophyllum nodosum was 5.6 μg/mL, 1181 μg/mL and 210.9, respectively.

Conclusion: Butanol fraction of Calophyllum nodosum can be a candidate as antiviral to DENV in future with high antiviral effect and no cytotoxic effect.

Keywords: antiviral drug, Butanol fraction of Calophyllum nodosum, Dengue virus serotype 2, Huh 7it-1 cell line
Chromatographic Fingerprinting of *Molineria latifolia*

Kunthasaya Akkarasirirathattana*, Siriphatr Chamutpong

*Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, PathumThani 12130, Thailand*

*Presenting author:*

Email: Kunthasaya_a@rmutt.ac.th, Phone: +6625491999

**ABSTRACT**

**Background:** *Molineria latifolia* (Dryand. ex W. T. Aiton) Herb. ex Kurz (Hypoxidaceae) is an herbaceous perennial species. It is widely exploited in traditional medicine in many countries. All parts of *M. latifolia* can be used for treatment many symptoms such as acne, freckles, melasma, inflammation, and wound. Therefore, it is very important and necessary to investigate their bioactive compounds for quality control of herbal medicine.

**Objective:** This present study aimed to evaluate the chromatographic fingerprinting of aerial and underground parts of *M. latifolia* using different solvents extraction.

**Methods:** Aerial and underground parts were separately extracted by ethanol, ethyl acetate and aqueous solution for fingerprinting analysis. Chromatographic fingerprinting was done using High Performance Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GC/MS).

**Results:** In HPLC fingerprinting, cinnamic acid and caffeic acid were found in ethanolic extract of aerial and underground parts, respectively, but quercetin was dependently found in only ethyl acetate extracts of underground part. In addition, nine compounds were identified by GC/MS fingerprinting, phytol (28.86 % RPA) and 9,12-octadecadienoic acid (28.86 % RPA) were successively found in ethyl acetate extract and ethanolic extract of aerial part. In underground part, 9,12-octadecadienoic acid was found in both ethyl acetate and Ethanolic extract (20.15 and 28.55 % RPA), and 9-octadecenoic acid was found in the aqueous extract with 27.29% RPA.

**Conclusion:** HPLC and GC/MS fingerprinting have been performed, which could be used for Identification and assessed for pharmacognostic specification in further study.

**Keywords:** *Molineria latifolia*, HPLC, GC/MS, Chromatographic Fingerprinting
Structure Modifications of Pinostrobin from Boesenbergia pandurata Roxb. Schlecht Extract

Andy Suryadi A. M.*, Retno Widyowati¹, Tri Widiandani², and Siswandoño²

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286;²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

*Presenting author
Email: monsteredans@gmail.com, Phone: +6282142220770

ABSTRACT

Background: Boesenbergia pandurata Roxb. Schlecht. (Zingiberaceae) is one of the ginger plants that has been identified to contain pinostrobin. It determined as an anti-inflammatory and analgesic activity through inhibition of COX2 enzymes.

Objective: The objective of this research was to obtain a pinostrobin derivative of acylation reactions between pinostrobin and acyl chloride derivatives.

Methods: The structure modifications of pinostrobin were obtained by Schotten-Baumann method through nucleophilic substitution reactions between pinostrobin and acyl chloride derivatives.

Results: The investigation of structure modifications of pinostrobin from this plant has demonstrated the present of pinostrobin acetate (1) and pinostrobin butyrate (2) based on the spectrometric analysis such NMR.

Conclusion: Pinostrobin acetate and pinostrobin butyrate which are derivatives of pinostrobin can be synthesized using the Schotten-Baumann method.

Keywords: Structure modification, Boesenbergia pandurata, pinostrobin, Schotten-Baumann.
Activity Banana Manurun (Musa paradisiaca Linn.) Stem Extract Against Staphylococcus aureus, Streptococcus pyogenes and Candida albicans In vitro

Lia Yulia Budiarti 1, Isnaini 2, NurIzatil Khasanah 3, Noorhasanah 3

1 Department of Microbiology, Medical Faculty of Universitas Lambung Mangkurat Banjarmasin; 2 Department of Pharmacology, Medical Faculty of Universitas Lambung Mangkurat Banjarmasin; 3 Student of general medical study program, Medical Faculty of Universitas Lambung Mangkurat

*Presenting Author
Email: isna.unlam@gmail.com, Phone: 085248715366

ABSTRACT

Banana manurun (Musa paradisiaca Linn.) is known as one of the typical plants of South Kalimantan. Part of banana stem will become waste after fruitful. However, the banana stalk is known to have active substances that can be utilized as a traditional medicine preparation. Manurun banana stems contain active substances such as alkaloids, flavonoids, saponins and tannins that act as antibacterial and antifungal. Aim. This study aims to test the inhibitory activity of some treatment of banana stem extract concentrations manurun against bacteria Staphylococcus aureus and Streptococcus pyogenes and Candida albicans fungi in vitro. This experimental study used 10 treatments of ethanol extract of banana stems manurun 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% and 50%, by control treatment that is antibiotic disk containing penicillin in test S. aureus and S. pyogenes as well as fluconazole in C. albicans. This study used paper disc diffusion method with measured parameter is the inhibit zone of each treatment on each test microbe. The results of this study have different mean of inhibitory zone of each treatment of banana stem extract at the same concentration on each bacteria and test fungi. (Anava test p < 0.005). There were significant differences in mean inhibitory effect between treatments tested at the same concentrations according to the LSD post hoc test (α 0.05). The treatment of 100% concentration extract gave the greatest inhibitory effect to all bacteria and test fungi. The conclusion is the inhibitory activity of banana manurun stem extract on Candida albicans is greater than the inhibitory activity on Staphylococcus aureus and Streptococcus pyogenes at the same concentration.

Keywords: inhibit zone, manurun banana stem extract, Candida albicans, Staphylococcus aureus, Streptococcus pyogenes
**The Activity of Sterculia quadrifida R.br Stembark against Hepatitis C Virus**

Maria AyuWandiraMoi Sola\(^1\)*, AditaAyu Permanasari\(^2\), Myrna Adianti\(^2,4\), Lidya Tumewu\(^2\), Aty Widyawaruyanti\(^2,3\), AchmadFuad Hafid\(^2,3\)

\(^1\)Graduate Master Student of Pharmaceutical Science Master Course Program, Faculty of Pharmacy, UniversitasAirlangga, Surabaya 60286, Indonesia;  
\(^2\)Institute of Tropical Disease, UniversitasAirlangga, Surabaya 60115, Indonesia;  
\(^3\)Department of Pharmacognosy and phytochemistry, Faculty of Pharmacy, UniversitasAirlangga, Surabaya 60286, Indonesia;  
\(^4\)Department of Health, Traditional Medicine Study Program, Faculty of Vocational, Universitas Airlangga, Surabaya 60286, Indonesia.

*Presenting Author  
Email: ayusola@yahoo.com, Phone: +62 85253156997

**ABSTRACT**

**Background:** Sterculia quadrifida R.br commonly known in East Nusa Tenggara, Indonesia as “Faloak”. It stembark has been used traditionally to cure liver disease. One of the cause active agent of liver disease is hepatitis C virus (HCV). Meanwhile, there is no information on the activity of this plant for anti-HCV.

**Objective:** This study was aimed to investigate the anti-HCV activity and cytotoxicity of extracts and fractions from S. quadrifida R.br stembark.

**Methods:** The stembark of S. quadrifida R.br was extracted using several different solvents. The stembark was gradually extracted using n-hexane, dichloromethane, and methanol. It was also extracted using 70% ethanol by ultrasonic assisted extraction method as well. In addition, it was extracted using water by decocta method. All samples were further analyzed for their anti-HCV activity using Huh7it cell and HCV JFH1a virus. While the cytotoxicity was determined by MTT assay. The most active extract was further separated by column chromatography and the fractions were tested for their anti-HCV activity and cytotoxicity.

**Results:** The anti-HCV assay results showed the water, 70% ethanol and methanol active against HCV with IC\(_{50}\) value of 6.06 µg/ml, 9.44 µg/ml and 10.39 µg/ml, respectively. Meanwhile, the hexane and dichloromethane extract were less active against HCV with IC\(_{50}\) value of 51.93 µg/ml and 179.31 µg/ml, respectively. Fractionation of water extract as the most active extract resulted seven fractions. Fraction 5 and 6 showed highest activity with IC\(_{50}\) value of 7.60 µg/ml and 8.87 µg/ml, respectively. Furthermore, the cytotoxicity of those two active fractions exhibited no toxicity with CC\(_{50}\) value of >2,000 µg/ml.

**Conclusion:** methanol extract, 70% ethanol extract, water extract, fraction 5 and fraction6 of water extract from S. quadrifida R.br stem bark had potential activity as anti-HCV.

**Keywords:** Sterculia quadrifida R.br, stembark, anti-HCV, JFH1a, cytotoxicity
ABSTRACT

Background: Hepatitis C virus (HCV) infection is a worldwide health problem that causes million deaths every year. Vaccine against HCV has not been discovered yet, and currently the combination of interferon-α (IFN-α) and ribavirin is a recommended therapy to treat HCV-infected patients. However, this combination therapy provokes severe side effects and quite expensive that probably not accessible for all HCV-infected patients worldwide. Therefore, development of new drugs for the treatment of HCV is an urgent need.

Objective: The objective of this study was to determine antiHCV activity of several plants belong to Rutaceae, Moraceae and Lauraceae family collected from Balikpapan, East Kalimantan.

Methods: Leaves and stem bark parts from eight different species of plants were extracted gradually using n-hexane, dichloromethane and methanol. AntiHCV activity test was conducted using Huh7.5 cells infected with HCV J6/JFH1. Each extract was treated to the infected cells by the concentration of 100 µg/ml. Cytotoxicity assay was done by MTT assay method.

Results: Five extracts out of 42 extracts showed more than 80% inhibition of HCV with more than 80% of viability. The extracts were dichloromethane and methanol extract of Melicope glabra leaves, methanol extract of Artocarpus sericicarpus leaves and stem bark, and methanol extract of Artocarpus dadah stem bark with % inhibition of 94.54 ± 0.00, 89.07 ± 1.55, 98.36 ± 0.77, 83.61 ± 4.64 and 99.45 ± 0.77, respectively. The extracts showed no cytotoxic effect.

Conclusion: Methanol extract of Artocarpus sericicarpus leaves and Artocarpus dadah stem bark showed antiHCV activities and potential to be further studied.

Keywords: antiHepatitis C Virus, plants extract, Artocapus, Melicope
Total Phenolic, Flavanoid and Antioxidant Activity of Stingless Bee Honey (Heterotrigona itama) from suburban and forest in Malaysia

Mohamad Zulhafiz Shafiq* 1, NorhaizanBintiMohd Esa1,2

1 Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia.
2Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.

*Presenting author
Email: zulhafizshafiq@gmail.com, Phone: +60194952452

ABSTRACT

Background: Stingless bee honey is a traditional dietary that contain compounds that responsible for antioxidant and redox activities such as phenolic acids and flavonoids. In Malaysia, stingless bee industries start to grow rapidly since 2015 and stingless bee honey itself been known as a superfood which contain high number of antioxidants and potential medical properties.

Objective: The study was intended to analyse and compare the antioxidant content in stingless bee Heterotrigona itama sp. honey collected from forest and suburban area in Malaysia.

Methods: The total phenolic content (TPC) was determined by Folin-Ciocalteu method while total flavonoid content (TFC) using aluminum chloride colorimetry method. The antioxidant activity was determined by 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) and ferric reducing antioxidant power assay (FRAP).

Results: The stingless bee honey samples from the forest have higher phenolic and flavanoid content with 520.663 ± 8.119 µg GAE/g and 443.25 ± 18.194 µg RE/g respectively. It also shows the highest antioxidant activities through multiple antioxidant assays. A positive correlation was determined between all the assays and the differences between antioxidant activities of all stingless bee honey samples were significantly found (P < 0.01).

Conclusion: Overall, all the stingless bee honey H. itama have shown a significant amount of polyphenol content as well as antioxidant properties with sample from forest clearly shown significantly higher total phenolic and flavonoid content as well as antioxidant activity compare to sample collected from suburban area.

Keywords: Stingless bee honey, Heterotrigona itama sp., forest, suburban, Antioxidant.
Isolation and identification of Bacillus spp. from stingless bee honey (Heterotrigona itama)

Fatin Aina Zulkhairi¹*, Norhasnida Zawawi¹,², Suriana Sabri³, Maznah Ismail¹, and Norhaizan Mohd Esa¹,⁴

¹Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, Malaysia; ²Faculty of Food Science and Technology, Universiti Putra Malaysia, Malaysia; ³Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, Malaysia; ⁴Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

*Presenting author
Email: fatinainaza@gmail.com, Phone: +60172386976

ABSTRACT

Background: Honey is a natural sweet substance produced by stingless bee honey and is well known for its nutritional and therapeutic uses. Despite of its usefulness, honey is known to contain certain microbes and is described as a reservoir for microbes. One of the microorganisms associated with stingless bee honey is Bacillus spp., which is recently known as new candidates for potential probiotic bacteria.

Objective: The aims of this study is to isolate and identify the strains and species of Bacillus spp. isolated from stingless bee honey, Heterotrigona itama of different origins.

Methods: Samples of stingless bee honey from 5 different places were collected from H. itama colonies and Bacillus spp. isolates were isolated from honey. The isolates were gram-stained and tested for catalase production. Gram-positive and catalase positive bacteria were selected for further identification. The 16S rRNA genes from extracted bacterial DNA were amplified with polymerase chain reaction using 16S specific primers (27F and 1492R). The obtained bacterial 16S rRNA genes were sequenced and deposited in GenBank.

Results: Results showed that bacteria isolated from honey range from 1.7 x 10¹ -6.4 x 10² CFU/ml. The selected bacterial isolates were indentified as Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus megaterium, Bacillus aryabatthai, and Bacillus pumilus.

Conclusion: Stingless bee honey has potential to be a source of new bacteria strains that can be good candidates for potential application as probiotics in stingless bees and also as probiotic cultures for consumption or as starter cultures in food industries.

Keywords: Stingless bee honey, Heterotrigona itama, Microorganisms, Bacillus spp.
Genotyping Study to Investigate Leprosy Distribution in the Past and Present Time in Indonesia

Dinar Adriaty¹, Ratna Wahyuni¹, Iswahyudi¹, Toetik Koesbardiati², Bimo Aksono¹, Cita Rosita Prakoeswa¹, Indropo Agusni¹, Shinzo Izumi¹

¹Institute of Tropical Disease, Universitas Airlangga, Mulyorejo Surabaya 60115, Indonesia
²Department Anthropology, Social and Politics Faculty Universitas Airlangga, Dharmawangsa Dalam Surabaya 60285, Indonesia
³Department Dermato and Venereology, Medical Faculty Universitas Airlangga, Prof. Moestopo 6-8 Surabaya 60285, Indonesia

ABSTRACT

Leprosy is a chronic disease caused by Mycobacterium leprae. Until now, it becomes a problem in Indonesia especially in the eastern part. According to the archaeological excavations results showed that leprosy already exist in the ancient time, this fact arises more question about how different the leprosy distribution between the past and present. Biomolecular study would be a great tool to explain the distribution of leprosy between them. The purpose of the research was performed diversity analysis of M. leprae based on repetitive sequences of TTC from ancient bones and leprosy patients in Indonesia.

PCR examinations performed on ancient bones from Lembata Island-Flores and Semawang-Bali, Indonesia, also 160 leprosy patients collected from Sumatera, Jawa-Bali, Kalimantan, Sulawesi and Nusa Tenggara and its TTC repeating pattern was seen in direct sequencing.

Genotyping result of ancient bone was obtained; Lembata has 13 copies of TTC and Bali 20 copies. While in patients are varies, from Nusa Tenggara 11 copies and Sumatera 34 copies, Jawa-Bali varies from 10-45 copies and mostly have 14 copies. From TTC repetitions showed that the distribution of disease has the same pattern, whether in the past or present time. Generally seen that the distribution derived from M. Leprae isolates in South American distribute to the east Indonesia and west Indonesia are from India.

Keywords: Genotyping, leprosy, past and present, Indonesia

Leprosy Study Group, Institute of Tropical Disease, Airlangga University Surabaya; Kampus C Airlangga University Jl. Mulyorejo Surabaya 60115; Phone: +62-31-5992445-6, 5965304; Fax: +62-31-5992445, 5965305; Website: www.itd.unair.ac.id
Assessing the Anti-Aging Capacity of *Acalypha indica* Associated with Malondialdehyde Levels in Old Sprague-Dawley Rats

Reganedgary Jonlean¹*, Rani Wardani Hakim²,³, Callen Zulkifli¹, Desak Gede Budi Krisnamurti²,³, Siti Farida²,³, Erni H. Purwaningsih²,³

¹Undergraduate student, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430; ²Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430; ³Drug Development Cluster IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430

*Presenting author
Email: reganedgaryjonlean@hotmail.com, Phone: +6289525862456

ABSTRACT

**Background:** Aging is a physiologic process characterized by degeneration of body functions and an increased oxidative stress level. Malondialdehyde (MDA) is an oxidative stress marker whose concentration will rise along with age. Increased level of MDA is associated with tissue aging and degeneration. Herbal plants are often used as alternative medications believed to contain many useful substances. *Acalypha indica* (Ai) is one of the herbal plants that can be found in many regions of Indonesia.

**Objective:** The aim of the study is to assess how the root of Aican affect MDA levels in relation with aging.

**Methods:** 28 old male Sprague-Dawley rats (age 20 – 24 weeks) are divided into 3 groups with an additional group consisting of young rats as negative control. The root of Ai extract was given orally to the the second group (treatment group) once a week for 28 days. On the last day of the experiment, the rats were sacrificed, and the internal organs and blood was collected as sample. MDA level was measured from the brain tissue, analyzed using one-way ANOVA.

**Results:** The means of MDA value are 3.776±1.083, 3.695±0.3947, 3.118±0.7845, and 3.704±0.4624 nmol/l, respectively for control group, treatment group, vitamin E group, and young rats group. Consumption of Ai extract is shown to able to decrease MDA levels in treatment group compared to the control group, but not statistically significant (p = 0.522).

**Conclusion:** *Acalypha indica* consumption apparently capable to decrease MDA levels in aged rats, but it requires further research.

**Keywords:** Malondialdehyde, Acalypha indica, oxidative stress, aging, brain.
Brain Derived Neurotrophin Factor (BDNF) Level in Aged Sprague Dawley Rats Brain after the Treatment of Centella asiatica Leaf Extracts

Indah Fitriani¹, Rani Wardani Hakim²,³, Nathaniel Aditya¹, Adisti Dwijayanti²,³, Desak Gede Budi Krisnamurti²,³, and Erni Hernawati Purwaningsih²,³

¹Undergraduate Student, Faculty of Medicine, Universitas Indonesia, Jakarta 10430; ²Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta 10430; ³Drug Development Cluster IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta 10430

*Presenting author
Email: fitrianiindah32@gmail.com, Phone: +6281314429857

ABSTRACT

**Background:** Functional decrease in learning and memory is one of the characteristics of the aging process. It has been known lower concentration of Brain Derived Neurotrophin Factor (BDNF) found on the brain, play a role in the phenomenon.

**Objective:** This study was designed to determine whether a herbal, Centella asiatica (CA) would increase the BDNF level on the aging brain tissue.

**Methods:** Male Sprague-Dawley rats aged 20-24 months and 4 weeks that used in the study were divided into: negative control (given aquadest), positive control (supplementation of Vitamin E), young rats as a comparison (4 weeks old), and treatment groups, which were given ethanol extract of CA leaf administered orally (150 mg/kg BW/week) for 28 days. At the end of the treatment, the rats were terminated and the brain BDNF levels were assessed. The data were analyzed by using one-way ANOVA.

**Results:** The results showed mean concentration of BDNF for negative control, positive control group, young and test groups were 44.09±3.854, 43.09±11.99, 65.88±13.46, and 30.2±12.33 mmol/ml, respectively (p<0.05 vs control group). The treatment group showed higher tissue BDNF level compared to all group. Interestingly, the BDNF level showed by the positive control group were found lower than the treatment group.

**Conclusion:** This result showed that supplementation of CA was effective in increasing brain level of BDNF, thus having a neuroprotective effect. However, the mechanism would be different with the Vitamin E. These results need further research to find out the mechanism of neuroprotective function exerted by CA.

**Keywords:** Centella asiatica, BDNF, Sprague-Dawley Rats, Aging
Anti-Oxidative Potential of *Acalypha indica* L. Root Extract on Brain-Derived Neurotrophic Factor Levels in Old Sprague-Dawley Rats

Callen Zulkifli\(^1\)*, Rani Wardani Hakim\(^2,3\), Reganedgary Jonlean\(^1\), Adisti Dwijayanti\(^2,3\), Desak Gede Budi Krisnamurti\(^2,3\), and Erni Hernawati Purwaningsih\(^2,3\)

\(^1\)Undergraduate Student, Faculty of Medicine, Universitas Indonesia, Jakarta 10430;\n\(^2\)Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta 10430;\n\(^3\)Drug Development Cluster IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta 10430

\*Presenting author
Email: callenlenn@gmail.com, Phone: +6285966795947

ABSTRACT

**Background:** In 2019, life expectancy in Indonesia is getting 69.8 years in estimation. It implies an increasing number of elderly and becomes a major problem in the health sector. Aging is a process that involves oxidative stress; however, it can be anticipated by the presence of brain-derived neurotrophic factor (BDNF).

**Objective:** The aim of this study is to investigate the effect of *Acalypha indica* L. (AI) as a notable medicinal plant to BDNF level changes as a marker of anti-aging.

**Methods:** This experimental study was conducted in 28 days with subjects used are 20-24 months of age. There were four groups identified: negative control, positive control (vitamin E 6 IU), treatment (AI 250 mg/kg BW), and comparative control (8-12 weeks of age rats). Data are collected from terminating all the rats and the brain tissues are further checked in the biochemistry laboratory. The test used for statistical analysis is one-way ANOVA.

**Results:** Compared with negative control group, levels of BDNF in brain tissues identified in the treatment group were increased, even though statistically insignificant (p-value = 0.6545). Nevertheless, BDNF level in the negative control group is still higher than shown in the positive and comparative control group.

**Conclusion:** These results show that AI provides anti-oxidative properties; it also implies that AI can be used to impede aging process. More studies are still needed to know the molecular mechanisms of action of AI that are particularly involved in increasing BDNF levels of brain tissues.

**Keywords:** *Acalypha indica* L., BDNF, aging, oxidative stress, anti-aging, anti-oxidative
The Anti-Oxidative Role of *Centella asiatica* on Brain Malondialdehyde Levels of Aged Sprague-Dawley Rats

Nathaniel Aditya¹*, Rani Wardani Hakim²,³, Indah Fitriani¹, DesakGede Budi Krisnamurti²,³, Siti Farida²,³, and ErniHernawati Purwaningsih²,³

¹Undergraduate Student, Faculty of Medicine, Universitas Indonesia, Jakarta 10430; ²Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta 10430; ³Drug Development Cluster IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta 10430

*Presenting author
Email: nathaniel.aditya@gmail.com, Phone: +6285727729780

ABSTRACT

**Background:** In 2050, the number of elderly with 65 years of age or more is estimated to be 1.5 billion. To better anticipate this imminent problem, a shift of paradigm, from chronological to biological aging, is needed. Aging is a multifactorial process closely related to oxidative stress; a phenomenon in which the rate can be indicated through its secondary metabolite level, malondialdehyde (MDA).

**Objective:** This study examines the effect of a well-known traditional medicinal plant used for its anti-inflammatory properties, *Centella asiatica* (CA), to brain MDA levels in aged Sprague-Dawley rats.

**Methods:** The aged male rats were divided into three groups: negative control, positive control (vitamin E 6 IU), treatment (CA leaves ethanolic extract 300 mg/kg), with additional one group consisted of young rats. Throughout 28 days, each rat was given the corresponding treatment. At the end, after termination, the brains were collected to be further studied using the Lipid Peroxidation (MDA) Assay Kit. One-way ANOVA is the choice of the statistical analysis method.

**Results:** We found that the level of MDA in the brain tissues of the treatment group rats had a lower value compared to that of the control group (3.412±0.6142 and 3.776±1.083 nmol/mL), although statistically insignificant (P=0.5683). Unquestionably, the MDA concentration in the positive control is still the lowest (3.118±0.7845 nmol/mL).

**Conclusion:** These results imply that CA has a positive anti-oxidative effect on aged rats which could hinder an aging process, if not prevent it.

**Keywords:** Aging, anti-oxidative, oxidative stress, malondialdehyde, MDA, *Centella asiatica*.
A Simple Method for Isolation of Citral Using Column Chromatography

Adlina Savira,1 Achmad Syahrani,1 Marcellino Rudyanto1,2*

1Faculty of Pharmacy, Airlangga University, Jl. Dharmawangsa Dalam, Surabaya, Indonesia.
2Institute of Tropial Disease, Airlangga University, Jl. Mulyorejo, Surabaya, Indonesia.

ABSTRACT

Background. Citral the main component of wintergrass (Cymbopogon citratus) oil. This compound has biological activities such as antibacterial, antifungi, analgesic, and antiinflammation. Citral also has importance for its use as starting material for the synthesis of Vitamin A. Due to the broad utilisation of citral, it is important to develop method of isolation which is relatively simple, low cost, but able to give pure citral in high yield.

Materials and method. Citral was isolated from commercially available wintergrass oil by simple column chromatography using silica gel as stationary phase. Elution was carried out in isocratic mode. Mobile phase was chosen among hexane – diethyl ether, hexane – ethyl acetate, and hexane – ethanol based on separation factor on thin layer chromatography. Optimum ratio of sample and stationary phase was also optimized based on isolation yield. Isolated citral was analyzed by gas chromatography – mass spectroscopy.

Results. The best separation factor on TLC was obtained from hexane – ethyl acetate (97:3) as eluent. The best yield (48.2%) was obtained when stationary phase was used at ratio 20:1 to sample.
Chitosan Nanoparticles as a Promising Topical Delivery System of Vitamin A Palmitate: Preparation and Penetration Property

Etik Mardliyati*, Sjaikhurrizal El Muttaqien, and Damai Ria Setyawati

Center for Pharmaceutical and Medical Technology, Agency for The Assessment and Application of Technology (BPPT), Jakarta 10340

*Presenting author
Email: etik.mardliyati@bppt.go.id, Phone: +6281314233415

ABSTRACT

Background: The therapeutic use of vitamin A palmitate as anti-aging ingredient in cosmetics is still limited because of its low effectiveness and poor chemical stability due to moisture, oxygen, and light exposure.

Objective: The purpose of this study was to develop topical delivery system for vitamin A palmitate by using chitosan as a wall material.

Methods: The encapsulation of vitamin A palmitate in chitosan was carried out by droplet formation via an oil-in-water emulsion, followed by droplet solidification using sodium tripolyphosphate as a cross-linking agent. The vitamin A loaded nanoparticles was characterized in terms of size, morphology, encapsulation efficiency, and ex vivo skin penetration profile.

Results: Spherical-shaped nanoparticles with diameter of ~136 nm was obtained, and the encapsulation efficiency of vitamin A in chitosan nanoparticles was around 98%. The skin penetration test by Franz diffusion cell revealed that penetration profile of vitamin A encapsulated in chitosan nanoparticles is greater than vitamin A non-encapsulated. Cream formulation of vitamin A encapsulated in chitosan nanoparticles showed better physical and chemical stability compared with cream of vitamin A non-encapsulated.

Conclusion: From these results, it can be concluded that chitosan nanoparticles show a promising topical delivery system of vitamin A.

Keywords: chitosan, nanoparticles, vitamin A palmitate, topical delivery system
**Metabolite Profiling and Predicted Anticancer Activities of Fruit Trees from Mt. Maliking, Laguna, Philippines**

Michael Russelle Alvarez\(^1\), Kimberly Delica\(^1\), Manolo Basingan Jr.\(^1\), Froila Marie Deniega\(^1\), Leonardo Cadiente III\(^1\), Rowell Abogado\(^2\), Isagani Padolina\(^2\), Gladys Completo\(^1\), Ruel Nacario\(^1\)

\(^1\)College of Arts and Sciences, University of the Philippines Los Baños, Los Baños, Laguna, Philippines.  
\(^2\)Pascual Pharma Corporation, Los Baños, Laguna, Philippines.  
*Presenting author  
Email: msalvarez1@up.edu.ph, Phone: 639171404862

**ABSTRACT**

**Backgrounds:** High-throughput bioactivity screening of plant extracts needs a robust method for characterization of the extracts, as well as a bioinformatics method to predict the bioactivity and correspondingly “filter” the required extracts needed to be tested.

**Objectives:** In this study, the metabolite profiles of leaves of fruit trees collected from the vicinity of Mt. Makiling, Laguna, Philippines. From the putative metabolite profiles, anticancer activities were predicted in silico.

**Methods:** Methanolic extracts of ground leaves of *Dillenia philippinensis*, *D. refferescheidia*, *Syzygium polycaphaloides*, *S. curannii*, *S. cuminii*, *Terminalia microcarpa*, *Microcos stylocarpa*, *Sandoricum koetjape*, *Achras zapota*, *Artocarpus altitlis*, *A. heterophyllus*, *Carissa carandas*, *Chrysophyllum cainito*, *Lansium parasiticum*, *Annona muricata*, *Garcinia mangostana*, *Canarium ovatum*, and *Antidesma bunius* were analyzed with a Xevo G2-S QToF UPLC-MS/MS system (data-dependent acquisition, positive ion mode, collision energy of 6-30 eV for low molecular mass and 30-70 for high molecular mass) using a typical gradient (5% to 95% acetonitrile+0.1% formic acid for 10 minutes) in a C\(_{18}\) column. Resulting data were analyzed using the GNPS platform (https://gnps.ucsd.edu) and mzMine 2.3 to putatively identify the compounds present in the corresponding chromatograms. Resulting list of compounds were predicted for anticancer activity against the NCI-60 database using CDRUG (http://bsb.kiz.ac.cn/CDRUG/). Putative compounds were ranked based on the software’s CDRUGscore to determine putative anticancer compounds in the extracts.

**Results:** Several compounds were detected from the methanolic extracts of these fruit trees, with the most commonly occurring being flavonoid and terpenoid compounds. Among the compounds detected, several compounds were predicted to have anticancer activity against the NCI-60 cell line panel, based on the algorithm by CDRUG. These compounds are: viguiestenin (log GI\(_{50}\)=-5.561, CDRUG score=1) from *Sa. koetjape*, karounidiol (log GI\(_{50}\)=-4.622, CDRUG score=1) from *A. altitlis*, triptolide (log GI\(_{50}\)=-7.931, CDRUG score=1) and gossypol (log GI\(_{50}\)=-5.529, CDRUG score=1) from *L. parasiticum*, annonacin (log GI\(_{50}\)=-5.863, CDRUG score=1) from *An. muricata*, cytochalasin a (log GI\(_{50}\)=-5.312, CDRUG score=0.939) from *M. stylocarpa*, and dihydroamorphigenin (log GI\(_{50}\)=-5.583, CDRUG score=0.814) and isouvaretin (log GI\(_{50}\)=-5.298, CDRUG score=0.799) from *Ar. heterophyllus*.

**Conclusion:** This study profiled the metabolites present in methanolic extracts of leaves of fruit trees found within the vicinity of Mt. Makiling, Laguna, Philippines. From the putatively identified compounds in these extracts, their anticancer activities against the NCI-60 cancer cell line panel was predicted in silico, with several compounds showing promising activities. To verify the anticancer activities of these extracts, anticancer activity screening need to be performed as well in the future.

**Keyword:** LC-MS/MS profiling, anticancer activity, plant extracts
ABSTRACT

Background: Dengue hemorrhagic fever is an infectious disease that is caused by dengue virus. This disease is spread by Ae. Aegypti mosquitoes. In Indonesia, 1.229 from 126.675 people that were diagnosed with DHF died in 2015 and this number keeps on increasing. There were several researches done that use natural substance as dengue antiviral, one of them is Cosmos caudatus. Cosmos caudatus contains active flavonoid that has antiviral effect.

Objective: The objective of this study to evaluate the mechanism of Cosmos caudatus n-hexane on DENV replication in vitro especially on targeting of DENV receptor.

Methods: In this study, we used 2 times the IC$_{50}$ value of 2.994 $\mu$g/ml on HUH7it-1 cell, human hepatoma cell line, to see the antiviral effect of Cosmos caudatus. We want to know the effect based on the time the extract was given which is at the receptor, in the entry step, post-entry step and both entry-post entry step. The inhibitory and toxicity effect were measured by focus assay and MTT assay, respectively.

Results: This study showed that the extract added on the receptor and entry-post entry step have anti-DENV effect. The treatment at the receptor and entry-post entry step exhibited 84% and 78.83% inhibition. N-hexane fraction of Cosmos caudatus leaves suppress DENV replication by binding with the receptor. This shows that the structure of Cosmos caudatus extract resembles the viral particle and has the ability to inhibit viral infectivity.

Conclusion: N-hexane fraction of Cosmos caudatus can be an alternative treatment as antiviral to DENV infection.

Keywords: Antiviral drug, n-hexane fraction of Cosmos caudatus, dengue virus serotype 2, HUH7it-1 cell line
In Vitro Evaluation of Antacid and Antiflatulent Activity in Ethanolic Extract Syrup of Clove Leaves (*Eugenia Caryophyllata* Thunberg)

Nurul Widi, Retno Widyowati, Herra Studiawan, Sukardiman*

*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy Universitas Airlangga, Surabaya*

*Presenting Author*
E-mail: maman_ht@yahoo.com, Phone: 081330458449/0818525342

ABSTRACT

**Objective:** Peptic ulcer has high prevalence in the world caused by high gastric acid. The sample of this study is ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) which has gastroprotective and antiulcer activities.

**Methods:** The pH of ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) and their neutralizing effects on artificial gastric acids were determined and compared with negative and positive controls, aluminium hydroxide and magnesium hydroxide. A modified model of Vatier’s artificial stomach was used to determine the duration of consistent neutralization effect on artificial gastric acids. The neutralization capacity in vitro was determined with the titration method of Wu model. Rezak method was used to test the antiflatulent activity by creating foam from sodium lauryl sulfate. The syrup was subsequently added, and the remaining height of foam formed was calculated.

**Results:** The results showed that both ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) have antacid and antiflatulence effects in vitro. Compared with the water group, ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) were found to possess significant gastric acid neutralizing effects. The duration for consistent neutralization of Ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) was significantly longer than that of water. Also, there are have antiflatulence effects by decrease of foaming in vitro model.

**Conclusion:** Ethanolic extract syrup of clove leaves (*Eugenia caryophyllata* Thunberg) were consistently active in the artificial stomach model and are suggested to have antacid and antiflatulence effects similar to the active control drugs.

**Keywords:** *Eugenia caryophyllata* Thunberg, antacid, antiflatulence, in vitro
Extract of *Ruta angustifolia* potentiated anti-hepatitis C activities with direct-acting antiviral agent.

Tutik Sri Wahyuni¹*, Adita Ayu Permanasari², Achmad Fuad¹, Aty Widyawaruyanti¹, Hak Hotta³.

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115; ²Institute of Tropical Disease, Universitas Airlangga Surabaya 60115 ³Department of Oral Vaccine and Drug Development, Kobe University Graduate School of Health Sciences, Kobe, Japan

*Presenting author
Email: wahyuni.tutiksri@yahoo.com, tutik.s-w@ff.unair.ac.id; Phone: 6281241435799

ABSTRACT

**Background:** Medicinal plants consist with many chemical substances that contribute to several bioactivities. Our previous studies were reported the anti-viral activities of *Ruta angustifolia* against hepatitis C virus. It still remains challenges to investigate anti-hepatitis C agents. Even though the current standard of care could improve the sustained virology response more that 90%, however the HCV related deaths is still high about 400,000 case annually due to the limited access to the direct acting antiviral drug(s) and the high cost. Moreover, there is no vaccine yet to prevent the eradication of HCV infection.

**Objective:** Our current study was observed the mechanism of *R. angustifolia* extract against hepatitis C virus and further determine the combination effect with direct acting antiviral agents.

**Methods:** Leaves of *R. angustifolia* were extracted with ethanol. A number of extract was dissolved in dimethyl sulfoxide and diluted in serial concentration of dubaco medium for antiviral assay. Antiviral activities were performed by *in vitro* culture using Huh 7.5it cells and infected with JFH1 strain. HCV-infected cells were treated with various combination of *R. angustifolia* extract and DAAs compounds. Cultures were incubated for 48 h and the virus infectivity in culture supernatant were determined. Mode of action analysis to observe the effect of extract in HCV life cycles were perform. Furthermore, the effect of extract on HCV NS3 protein was detected by western blot analysis.

**Results:** Mode of action analysis was shown that extract of *R. angustifolia* inhibit HCV dominantly in the post entry step and reduced the HCV NS3 protein level up to 80%. The combination of *R.angustifolia* extracts with simeprevir or telaprevir was increase the anti-HCV percentage inhibition.

**Conclusion:** Extract of *R. angustofolia* exhibit anti-HCV activities, decrease HCV NS3 protein level and revealed a potentiated activity with direct acting antiviral drugs. There results indicated that extract of *R. angustifolia* may a good candidate to develop anti-HCV agents.

**Keywords:** *Ruta angustifolia*, Hepatitis C virus (HCV), Medicinal plants.
Chemoprevention Effect of *Curcuma aeruginosa* Roxb. On Breast Histopathology of DMBA Induced Rat

Kurnia Agustini¹, Soufa Malita², Sri Ningsih¹, Asri Sulfianti¹, I Made Artika²

¹Center for Pharmaceuticals and Medical Technology, Agency for the Assessment and Application of Technology (BPPT), PUSPIPTEK, Serpong – Indonesia; ²Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia

*Presenting Author  
Email: kurnia.agustini@bppt.go.id, Phone: 0217560707

**ABSTRACT**

Temu ireng (*Curcuma aeruginosa* Roxb) is one of Indonesian Medicinal Plant known have many pharmacological activities. Empirically, Temu Ireng Rhizomes used for anthelmintic and depurative, also used to treat colic, asthma, cough, obesity and rheumatism. This research was conducts to investigate the chemoprevention effects of Ethanolic Temu Ireng Extracts (ETIE) on breast histopathology of 7,12-Dimetilbenz [α] anthracene (DMBA) induced rats. 48 female white rats were divided into 6 groups namely normal control; negative control treated only DMBA; positive control treated with commercial herbal medicine; and three groups of ETIE in three dose variations (40mg/200g, 80mg/200g and 160mg/200g BW). ETIE treatment was given from 2 weeks before DMBA induction until the end of the experiment (21 weeks). The parameters measured were the number of tumors, weight, volume, incidence and tumor severity based on histopathology test of Haematoxylin-Eosin staining. Results showed that the quantity, weight, volume and tumor severity of ETIE treatment group were significantly (P<0.05) lower than negative control and positive control. ETIE treatment at 80mg/200gBW give the best effective tumor inhibition by reduce tumor incidence 50%. From these results we can conclude that ETIE has potential as chemoprevention.

**Keywords:** breast tumor, Chemoprevention, *Curcuma aeruginosa* Roxb, histopathology, HE staining.
Anti-Hepatitis C Virus Activity of Methoxyflavone isolated from *Melicope latifolia* Leaves

Aty Widyawaruyanti1,2*, Lidya Tunewu2, Achmad Fuad Hafid1,2, Tutik Sri Wahyuni1,2, Adita Ayu Permanasari2, Myrna Adianti2,6, Abdul Rahman1, Maria Inge Lusida2, Soetjipto2, Hiroyuki Fuchino3, Nobuo Kawahara4, Chie Aoki3,5 and Hak Hotta3

1Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia; 2Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia; 3Department of Oral Vaccine and Drug Development, Kobe University Graduate School of Health Sciences, Kobe, Japan; 4Research Center for Medicinal Plant Resources, National Institutes of Biomedical Innovation, Health and Nutrition, Tsukuba, Ibaraki, Japan; 5Department of International Health, Kobe University Graduate School of Health Sciences, Kobe, Japan; 6Department of Health, Traditional Healers Study Program, Faculty of Vocations, Airlangga University, Surabaya, Indonesia

*Presenting Author
Email: aty_ww@yahoo.com; aty-w@ff.unair.ac.id; Phone: +62 8113404171

ABSTRACT

**Background**: Development of complementary and alternative drugs for treatment of hepatitis C virus (HCV) infection is greatly needed. In our previous study we reported that ethanol extract of *Melicope latifolia* leaves possessed anti-HCV activities with IC50 value of 3.5 µg/ml and 2.1 µg/ml, respectively, against J6/JFH1-P47 and J6/JFH1-P1.

**Objectives**: In this study, bioactivity guided isolation of *M. latifolia* extract was performed to purify and isolate its active constituents against HCV.

**Methods**: Anti-HCV activities were tested using in vitro culture cells of hepatocyte cell line Huh 7.5 and HCV genotype 2a (J6/JFH1). Ethanol extract of *Melicope latifolia* leaves were subjected to separation by chromatographic method and then the chemical structures of isolated compounds were established on the basis of mass and 1D and 2D nuclear magnetic resonance spectral data, as well as comparison with reported data.

**Results**: A prenyloxy flavone identified as 5,4'-dihydroxy-7-prenyloxy-3,3',8-trimethoxyflavone (1) and two methoxy flavones, 5,3'-dihydroxy-3,7,8,4'-tetramethoxyflavone (2) and 5-hydroxy-3,7,8,3',4'-pentamethoxyflavone (3) were isolated from ethanolic extract of *M. latifolia* leaves. Anti-HCV activities revealed that 5,4'-dihydroxy-7-prenyloxy-3,3',8-trimethoxyflavone (1) inhibited HCV J6/JFH1 with IC50 value of 6.7 µg/ml and CC50 of 19.3 µg/ml and showed dominantly to inhibit HCV in the entry step. While, 5,3'-dihydroxy-3,7,8,4'-tetramethoxyflavone (2) and 5-hydroxy-3,7,8,3',4'-pentamethoxy flavone (3) did not exert significant anti-HCV activities.

**Conclusions**: 5,4'-dihydroxy-7-prenyloxy-3,3',8-trimethoxyflavone showed antiviral activities against HCV J6/JFH1 mainly during virus inoculation or entry step inhibition.

**Keywords**: *Melicope latifolia*, prenyloxy flavones, anti-HCV, J6/JFH1
Optimization of Clinacanthus nutans Biodegradable Patch for Analgesic

Em-on Chaiprateau1*, Chalermsak Thavornwat2

1Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Pathum Thani 12130, Thailand;
2Department of Industrial Engineering, Faculty of Engineering, Rajamangala University of Technology Thanyaburi, Pathum Thani 12130, Thailand

*Presenting author
Email: emon_c@rmutt.ac.th, Phone: +66808666091

ABSTRACT

Background: The whole Clinacanthus nutans (C. nutans) is a traditional medicinal herb grows in a tropical Asian country and popular used to treat inflammatory like pain, contusion, gout, rheumatism, strains and sprains of injuries. Current pharmaceutical products for analgesic are topical creams and ointments, which are inconvenience to use.

Objective: The purpose of the study was to optimize and evaluate the C. nutans-based biodegradable patch for analgesic using design of experiment (DoE) by Minitab version18 program.

Methods: Fifty-four formulation from full factorial design use to determine 3 factors and 3 levels compose of the different concentration (2%, 4%, 6%) of sodium alginate and aloe vera act as natural film former and type of terpenes act as permeation enhancers (eugenol, anethole, limonene). The Physicochemical properties including weight variation, thickness, strength, % elongation, pH, mucoadhesive time were investigated.

Results: The results demonstrated that combination of 6% of sodium alginate and anethole were the best max stress, % elongation (R2 adj > 90%, p<0.05). In conclusions, there were statistically significant expressed for the optimum concentrate of sodium alginate (ALG-Na) a natural film former is 6% (p<0.05) and type of permeation enhancer is anethole (p<0.05). Accelerated stability test was found that the weight and thickness of patch were did not different statistically significant (p<0.05).

Conclusion: It is a favorable physicochemical and cost profile for biodegradable dermal analgesic patches commercial product further.

Keywords: Clinacanthus nutans, Pain, Patch, Biodegradable, Analgesic, DoE
Antimicrobial Activity of Selected Malaysian Mushrooms against Staphylococci and Streptococci Species

Samer Al-Dhalli1*, Pavithira Paniker Ravandran1, Jiyauddin Khan 1, Brian Teo Sheng Xian 1, Sri Budiasih1, Mohammed Kaleemullah1, Rasha Saad1, Heyam Saad Ali2, Gamal Osman Elhassan1, Ram Sahu4, Fadli Asmani1, Eddy Yusuf 5

1School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; 2Department of Pharmaceutics, Dubai Pharmacy College, Dubai, United Arab Emirates; 3Unaizah College of Pharmacy, Qassim University, Qassim, Kingdom of Saudi Arabia; 4Department of Pharmacology, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India; 5International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: samer@msu.edu.my Phone: +603-55216488

ABSTRACT

Background: One of the biggest problems in the developing world is the proliferation of treatable bacterial infections which run unchecked through populations due to lack of access to medications and medical treatment. Therefore, suitable action must be taken at the right time to combat this problem. The use of mushrooms as a functional food and a phytomedicine is gaining popularity, and they are widely used in complementary medicine.

Objectives: The aim of this research is to evaluate the antimicrobial activity in Lentinula edodes, Pleurotus ostreatus and Agaricus bisporus against Staphylococcal and Streptococcal bacteria.

Methods: The mushroom was dried and grounded into powder. The extracts of powder were prepared through cold maceration process. The solvent was removed through evaporation and then the extract was tested for its antimicrobial activity against Staphylococci and Streptococci microorganisms. In-vitro antibacterial screening of the extracts by Disc Diffusion Method was done on Staphylococci and Streptococci microorganisms.

Results: The chloroform extract of Shiitake mushroom exhibited maximum zone of inhibition against Streptococci species with a 14 mm diameter. The moderate activity of acetone extract against Streptococci species was observed with a 12 mm diameter. The chloroform extract showed broad spectrum of antibacterial activity against the tested bacterial pathogens than other solvents. The MIC assay showed that 50 mg/ml of acetone extract and 12.5 mg/ml of chloroform extract of the shiitake mushroom have antibacterial activity with reliable indication of the concentration of drug required to inhibit the growth of microorganism.

Conclusion: The results shows that Shiitake mushroom may serve as leads for the development of new antibiotic against the Staphylococci and Streptococci Species.

Keywords: Lentinula edodes, Pleurotusostreatus, Agaricusbisporus, Staphylococci and Streptococci
Method validation of contact and immersion TLC-Bioautography for determination of streptomycin in shrimp

Febri Annuryanti¹, Iftitahatur Rosyida², Aprilita², Isnaeni¹*

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286
²Undergraduate student, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

*Presenting Author
Email: isna.yudi@gmail.com Phone: +6281331021303

ABSTRACT

Background: Shrimp is one of the export commodities that generate significant economic values. Uncontrolled farming makes the shrimp susceptible to disease, so generally farmers use antibiotics to prevent undesirable risks. An antibiotic residue on food can be disrupted to human health, especially as it may contribute to antibiotic resistance through the food chains. Therefore, a rapid and perfect method of analysis is needed to detect the antibiotic residues. The TLC-bioautography method can be used to determine the levels of antibiotics that available in samples.

Objective: In this research, streptomycin analysis was done by using both contact and immersion TLC-Bioautography method. From the validation method of this study it will be known the most effective and efficient method in determining the level of streptomycin.

Methods:
Contact TLC-Bioautography
The test solution was spotted on the TLC silica gel plate GF 254 then was eluted with KH₂PO₄ 7.5%. The TLC plate was dried and attached to the surface of agar. Followed by inoculation of agar with Escherichia coli (5μL/15 mL) test bacteria in a petri dish and stored in the refrigerator for 3 hours. The TLC plate was then removed and the petri dish was incubated at 37ºC for 24 hours. The inhibition zone was observed and measured.

Immersion TLC-Bioautography
The test solution was spotted on the TLC silica gel plate GF 254 then was eluted with KH₂PO₄ 7.5%. The TLC plate was dried and coated with 15 mL of agar medium inoculated with Escherichia coli (5μL/15 mL) to form a thin film at 50ºC. The TLC plate was stored in sterile petri dishes then incubated at 37ºC for 16-18 hours. The plates were sprayed with 0.25% methyl thiazole tetrazolium then incubated at 37ºC for 4-6 hours. The white-yellowish inhibition zones then observed.

Results: It was found that the resolution (Rs) value of contact and immersion TLC-Bioautography was 1.83. The LOD and accuracy result of contact TLC-Bioautography were 30 ppm and 80%, respectively. Whereas the LOD and accuracy result of immersion TLC-Bioautography were 20 ppm and 97%, respectively.

Conclusion: Immersion method gave better validation parameter result than contact method. Nevertheless, both methods still meet the validation parameter requirements and can be used in the determination of streptomycin levels in shrimp samples.

Keywords:TLC-Bioautography, immersion, contact, streptomycin, shrimp.
Aphrodisiac Activity of Ethanol Extract of Three Pepper Plants from Piperaceae Family

Idha Kusumawati¹, Rohman Hadi¹, Syailendra Mahatmaputra¹, Helmy Yusuf¹ and Abdul Rahman¹

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Jl. Dharmawangsa Dalam 60286, Surabaya, Indonesia

*Presenting Author
Email: idha-k@ff.unair.ac.id, Phone: 08123543490

ABSTRACT

Pepper plants are species belong to the Piperaceae family that have many pharmacological activities and have traditionally been widely used for various therapies, one of which is as an aphrodisiac. In this research, aphrodisiac activity study of three pepper plant, *Piper nigrum, Piper retrofractum, and Piper cubeba* were elucidated. The level of piperin, the active compound of plants from the family Piperaceae, also determined in the ethanol extract of those three fruits of pepper plant. The results showed that the extract of *Piper retrofractum* ethanol extract had the highest piperin content and the highest aphrodisiac activity.

**Key words:** Aphrodisiac, Piperin level, Piperaceae, *Piper nigrum, Piper retrofractum, and Piper cubeba.*
ABSTRACT

Background: Entamoeba histolytica is a protozoan parasite and the causative agent of amebiasis. It is estimated approximately 1% of humans are infected with E. histolytica, resulting in an estimate of 100,000 deaths annually. Current treatment for amebiasis infection are known to have side effect such as nausea, headache, urine became dark color, and etc. Therefore, new medicine is needed to overcome these side effects and treatment using natural product is a potential alternative. L-cysteine biosynthetic pathway is essential for various cellular activities, including the proliferation and anti-oxidative defense of E. histolytica. Since the pathway, consisting of two reactions catalyzed by serine acetyltransferase (SAT) and cysteine synthase (CS, O-acetylserine sulfhydrylase), does not exist in humans, it is a rational drug target against amebiasis.

Objective: To determine the antiamebiasis activity of several plants by enzymatic assays.

Methods: Plant collected from Balikpapan Botanical Garden in East Kalimantan, extracted gradually using solvent hexane, dichloromethane, and methanol. Screening of these plant extracts done with two enzymatic assay methods, which were SAT1/CS3 couple assay and CS single assay. For SAT1/CS3 couple assay prepare master mix of distilled water, Na2S, DTT, L-Serine, and SAT1/CS3 enzyme, add with acetyl-COA and Tris Buffer. For CS3 single assay make master mix of distilled water, OAS, and CS3 enzyme, add Na2S and Tris Buffer. For those assays, add each master mix with extract and incubate 37°C 10 minutes then add acetic acid and nynhidrin reagent, incubate 95°C, and read absorbance at 560 nm.

Results: The results from 114 extract Balikpapan plants there are 32 extract showed inhibition >75% for SAT/CS couple assay, and 13 extract showed inhibition >75% for CS single assay. Six species plants showed inhibition >75% on SAT/CS and CS enzymatic assay.

Conclusion: Six species plants showed activities on both assay and potential as a candidate to be further examine as an antiamebiasis drugs remedies and will be continued for fractionation and purification.

Keywords: antiamebiasis, enzymatic assay screening, SAT1/CS3 coupled assay, CS3 single assay
Molecular Docking Study of Catechin and Epicatechin conjugated with fatty acid as a Hepatitis B Virus Replication Inhibitor

Susi Kusumaningrum1* and Raodatul Jannah1

1Center of Pharmaceutical and Medical Technology, Agency for Assessment and Application of Technology, Building 610-611 Puspiptek Area South Tangerang 15314, Indonesia, 021-7560536

*Presenting author
Email: susi.kusumaningrum, Phone: +628128861031

ABSTRACT

Background: Catechin and its derivatives have been known for long time to have anti-oxidant, anti-cancer, anti-diabetic, anti-bacterial, anti-inflammation and anti virus. The hepatitis B virus (HBV) infection is a worldwide health problem and afflicts more than 350 million individuals world-wide. By disrupting the role of HBV core proteins, viral replication will be inhibited.

Objective: This simulation aimed to predict the interaction between Catechin and Epicatechin conjugated with fatty acid with virus core protein causing disruption of capsid formation and inhibiting its replication.

Methods: The structure of Catechin and Epicatechin conjugated with fatty acid were prepared using MarvinSketch. The interaction and binding of ligands – protein was done and visualized using Molegro Virtual Docking (MVD) 6.0. The 3D stable conformation of molecule analyzed to view the minimum energy and interactions that occurred. The coordinate docking was done at the same coordinate as the previously docked reference ligand position and was validated.

Results: From the results it was known that epicatechin-C12 and epicatechin-C18 formed the most stable affinity bond with amino acid residues of viral core proteins. It is predicted that epicatechin-C12 and epicatechin-C18 has potency as lead compound to find a new anti HBV candidates for possible therapeutic agents.

Conclusion: Epicatechin-C12 and epicatechin-C18 has potency as lead compound for possible therapeutic agents HBV.

Keywords: Catechin, Epicatechin, Fatty acid, Core protein, HBV, Molecular docking.
Hepatoprotective Activity Ethanol Extract of *Curcuma heyneana* Rhizome Against Isoniazid and Rifampin-Induced Liver Injury in Rat

Chintya Monica Thampatyi, Mariannei*, Urip Harahap1

1Department of Pharmacology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia

*Presenting author
Email: marianne80@usu.ac.id, Phone: +6281361204672

ABSTRACT

**Background:** Isoniazid (INH) and rifampin (RIF) are antituberculosis drugs that can cause hepatotoxicity.

**Objective:** This study investigated the hepatoprotective activity of the ethanol extract of *Curcuma heyneana* rhizome which is induced by INH and RIF.

**Methods:** Wistar rats were divided into 7 groups. All animals were induced by INH at the dose of 50 mg/kg and RIF at the dose of 100 mg/kg for 15 days along with administration of extracts or comparative substance. Group I is negative control which was given sodium CMC only; group II to V are treatment groups, which were given ethanol extract of *C. heyneana* rhizome at doses of 5, 25, 125, and 625 mg/kg respectively, group VI is positive control which was given catechin and group VII is normal control. On the 16th day, the rats were sacrificed, blood and liver were collected for assessment enzyme alanine transaminase (ALT), aspartate transaminase (AST) and histopathology.

**Results:** Administration of ethanol extract of *C. heyneana* rhizome at the doses of 25, 125 and 625 mg/kg significantly inhibit the elevation of AST and ALT compared to negative control (p<0.01). They also have same effect as positive control and normal control (p≥0.01). Moreover, extracts could effectively reduce the pathological tissue damage.

**Conclusion:** This result suggests that ethanol extract of *C. heyneana* rhizome at the doses of 25, 125 and 625 mg/kg effective as hepatoprotector.

**Keywords:** *Curcuma heyneana*, hepatoprotective, rifampin, isoniazid
Evaluation of Antibacterial Activity of Different Extract of *Ipomoea aquatic* Leaves against *Staphylococcus aureus* and *Streptococcus pyogenes*

Mohamed Rasny Mohamed Razik¹, S. Angielina¹, Reyadh Al-Rashidi¹, Samer Al-Dhalli¹, Jiyauddin Khan¹, Kiran Chanabasappa Niluga¹, Santosh Fattepur¹, Kaleemullah, M¹, Shariq Baber¹, Chen Jie¹, Fadli Asmani¹, Eddy Yusuf²

¹School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; ²International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: mohd_rasny@msu.edu.my, Phone: +603-55216488

**ABSTRACT**

**Background:** The skin is the largest organ in the body and can be vulnerable to various microbial infections. Although antibiotics are clinically proven to be useful in the treatment of bacterial skin infections, they are largely subjected to antibiotic resistance and adverse effects. This has led to the screening of several medicinal plants for their potential antimicrobial activity since they are less expensive, has reduced occurrence of adverse effects and widespread availability.

**Objective:** The aim of this research will focus on evaluating the antibacterial activity of different extracts of *Ipomoea aquatic* leaves against *Staphylococcus aureus* and *Streptococcus pyogenes* that causes skin infections.

**Methods:** Leaves were extracted separately with 95% methanol and 95% ethanol using maceration process. Phytochemical screening was done for each extract and the minimum inhibitory concentration (MIC) was determined for each extract against both bacteria using 10 different concentrations ranging from 10 mg/ml up to 100 mg/ml via disc diffusion method in triplicates. Two concentrations above the MIC from each extract were selected and antibacterial assay of the different extracts against the two bacteria respectively was performed using disc diffusion method in triplicates.

**Results:** MIC for methanolic extract against both bacteria was 10 mg/ml, while MIC for ethanolic extract was 10 mg/ml against *Staphylococcus aureus* and 30 mg/ml against *Streptococcus pyogenes*. Methanolic extract of the plant at a concentration of 90 mg/ml and 100 mg/ml was statistically significant against *Streptococcus pyogenes* with a significance value of 0.00 ($p<0.05$), with 100 mg/ml having larger mean inhibition zone of 17.00 mm ± 0.00 mm than 90 mg/ml (14.33 mm ± 0.58 mm). Statistical analysis was performed using one way ANOVA (Tukey’s Test).

**Conclusion:** Both methanolic and ethanolic extract of the leaves has positive antibacterial activity against both *Staphylococcus aureus* and *Streptococcus pyogenes* at different concentrations.

**Keywords:** *Ipomoea aquatic*, *Staphylococcus aureus*, *Streptococcus pyogenes*
Gendarusin A Isolate from *Justicia gendarussa* Burm.f Leaf as Anti-HIV

Bambang Prajogo EW
Pharmacognosy and Phytochemistry Department, Faculty of Pharmacy Universitas Airlangga, Indonesia

ABSTRACT

Many extracts from *Justicia gendarussa* Burm.f leaves had already been screened to check the virus growth level of the blood plasma of HIV patient from Dr Soetomo Regional General Hospital. The extract of *J. gendarussa* leaves had already known that impedes the reverse transcriptase HIV type 1, and also the main content is gendarusin A, besides gendarusin B, JGF1, JGF2 and the recently detected of JGF3.

At the first time the extraction and fractionation done by 3 models that pose methanol absolute solvent, 70% of methanol and 70% of ethanol with the deliverance of alkaloid. After that, each of fraction sample got incubate in the plasma of HIV patient with 3.6 $10^6$ litre copied during 1 hour with content of 1.64ppm, 4.1ppm, 8.2ppm, 16.4ppm and 41.0 ppm. Alter the incubation do the examination by using the Nucli sens machine is the combination between PCR and Elisa to avoid the direct contact with the very pathogen virus.

The obtained result is that the sequence of activities from the most potential into the weakest among other each 1.64 ppm > 4.1 ppm > 8.2 ppm > 16.4 ppm > 41.0 ppm, with the value of obstacle 0.62 $10^6$, 1.4 $10^6$, 1.6 $10^6$, 2.4 $10^6$ and 5.2 $10^6$cell/ml. The conclusion is that the highest anti-HIV activities come from the isolate gendarusin Ain1.64 ppm concentration. Next after doing the linear regression of $y=-3.063x+81.37$ obtained IC$_{50}$ 10.24 ppm.
Cytotoxic effect of Binahong (*Anredera cordifolia* (Ten.) Steenis) in 3T3-L1 cell line

Asri Sulfianti¹*, Churriyah¹, Nuralih¹, Mayriska¹

¹Center of Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology, Indonesia 15311

*Presenting author
Email: asrisulfianti88@gmail.com, Phone: +6285885266350

**ABSTRACT**

**Background**: The determination of cytotoxicity with cell cultures could be an important complementary method to get more information about a general basal toxicity of a substance in an organism during the course of an acute toxicity experiment. We employed cytotoxicity test of the 3T3-L1 pre-adipose cell line treated by Binahong (*Anredera cordifolia* (Ten.) Steenis) leave extracts. Binahong is one of the Indonesian famous medical plant. The active compounds made this plant widely used as antidiabetic, antibiotic, antioxidant, antiinflamation, and anticancer. A study found, the methanol extracts of Binahong leaves are able to decrease the blood glucose in in-vivo animal model. But, the study of this herbs as antidiabetic using cell line model is still rare.

**Objective**: Present study is aimed to investigate the cytotoxic effect of Binahong leaves extracts in 3T3-L1 adipose cell line. As a quantitative marker of cytotoxic effects, we determined the inhibition concentration for a fifty per cent reduction of the cell number per culture (IC50).

**Methods**: A 70-80% of confluent cells were treated with 6 doses of Binahong extracts (31.25; 62.5; 125; 250; 500;1000 ppm). As a positive control, other cells group was treated by Metformin. Cytotoxic activity was performed by using [3-[4, 5-dimethylthiazol-2yl] 2, 5 diphenyltetrazolium bromide] MTT assay. Water soluble yellow MTT is metabolized by the metabolically active cells to the water insoluble purple formazan. The resultant product is quantified by spectrophotometry using a plate reader at 570 nm.

**Results**: The reports shown, IC50 of the Binahong leaves extract was 500 ppm (µg/ml) dose. The highest dose (1000 ppm) of this extract caused 92% cell death (letal dose). As comparison, all doses of Metformin as positive control were safe.

**Conclusion**: A relatively non-toxic doses of Binahong extract in 3T3-L1 pre-adipose cell line were less than 500 ppm. Therefore, in the next study, 62.5; 125; 250 of ppm extracts can be used to determine the antidiabetic activity in pre-adipose cell line.

**Keywords**: Binahong, IC50, toxicity, cell line, MTT assay.
Virtual Screening of Quinone Compounds as Hepatitis B Virus Replication Inhibitor

Firdayani*

Center on Pharmaceutical and Medical Technology-BPPT, Laptiab Puspiptek Tangerang Selatan, Indonesia

*Presenting author
Email: firdayani@bppt.go.id, Phone: +628159431762

ABSTRACT

Background: Quinones are secondary metabolites isolated principally from plants and having an aromatic (hexacyclic saturated) di-one or di-ketone system. Naturally occurring quinones are widely distributed and include benzoquinones, naphthoquinones, anthraquinones, and polyquinones. They exhibit numerous biological activities such as neurological, antibacterial, antiplasmodial, antioxidant, trypanocidal, antitumor, and anti-HIV.

Objective: It was predicted that quinones could act as hepatitis B virus replication inhibitor. One of the main factors in virus assembly in replication cycle is core protein (Cp) so it suitable to use as one of therapeutic target for hepatitis B agent.

Methods: It was performed the virtual screening to discover and choose a lead compound of hepatitis B virus inhibitor from Quinone groups and design the modified structure to get a predicted structure of antihepatitis B agent. Docking simulation and scoring were done Molegro Virtual Docker 6.0 program. The macromolecule target was protein core of hepatitis B virus and downloaded from Protein Data Bank (PDB ID: 5WRE)

Results: The core structure of Quinone compounds do not show a good score when docked into core protein of HBV as receptors. But when added the other functional groups on them, there are increases in docking scores.

Conclusion: The structure of Quinone compound could predict as a lead which has potency to be developed and evaluated further as an antihepatitis B agent. To acquire a potential anti-hepatitis B agent candidate from the Quinone group it is necessary for modification or optimization by adding other functional groups at the basic framework of Quinone.

Keywords: virtual screening, quinones, hepatitis B virus, core protein, molecular docking
In Silico Screening of Rocaglamide’s Action Mechanism to Induce Apoptosis on Chronic Myeloid Leukemia Pathway

Deasy Vanda Pertiwi¹, BS Ari Sudarmanto²

¹Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta; ²Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta

*Presenting author
Email: deasy.pertiwi@pharm.uad.ac.id,

ABSTRACT

Background: Rocaglamide is a compound with benzoafuran derivative that comes from traditional Chinese medicinal plant Aglaea sp (Chinese boyfriends). In traditional Chinese medicine, rocaglamide known as inhibitor of cancer by inducing apoptosis. However, that specific target and mechanism is not yet known.

Objective: The aim of this research was to indentify the mechanism of action and specific target of rocaglamide to inhibit of leukemia by computational. Based on Chronic Myeloid Leukemia Pathway, proteins that are involved in apoptosis pathways are PI3K, Bcl-xl, PKB/Akt, and NFκB.

Methods: Software of Molecular Operating Environment (MOE) 2010.10 version was used in this research. The selection of the target protein is performed based on the resolution and a complex with native ligand. PDB file of protein is prepared by 3D protonation and the addition of of charges by AMBER99 method. Preparation of native ligand and rocaglamide is performed with the semiempirc AM1 method. The ligand then used for docking at the binding site of protein targets, using the Alpha algorithm PMI and the Scoring function of London dG. Rocaglamide scoring results then compared with the ligand native and plotting it against pIC50.

Results: Results of docking and scoring values indicate that NFκB p65, PI3K, and PKB/Akt1 have a better affinity to form a binding with rocaglamide and its derivatives compare with PME/Akt2, Bcl-xl, and NF-kB p50 because score of S gives a lower value compare with ligand standard.

Conclusion: NF-kB p65 subunit is the most potent target of rocaglamide and its derivatives because it gives the greatest value of r2 i.e. 0.491. The amino acid that gives an important role in the binding of rocaglamide on p65 receptor was Gln 220, Lys 221, His181, and Gln 29.

Keywords: docking, leukemia, apoptosis, rocaglamide
Identification of Standard Parameter of Mindi Leaves (*Melia azeredach* L.) Based on Indonesian Herbal Pharmacopeia for Anti-cancer Herbal

Sukardiman*, Devky Rivina, Lusiana Arifianti, Herra Studiawan

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya

*Presenting Author
Email: maman_ht@yahoo.com, Phone: 081330458449/0818525342

ABSTRACT

Mindi (*Melia azeredach* L) has a potency to be developed as anti-anticancer agent. The raw material is affected by many factors such as cultivation, post harvest and processing. Standard parameters were used to ensure safety, efficacy and quality of the product. This study identified some of standard parameters based on Indonesian Herbal Pharmacopeia that may be useful as guidelines to choose mindi leaves for anti-cancer herbal.

**Keywords:** standard parameter, raw material, *Melia azeredach* L.
Antidiabetic Activity of Diterpene Lactone Fraction of “Sambiloto” (*Andrographis paniculata* Nees.) on Mice (*Mus musculus*) Induced by Alloxan

Rani Maylana, Wiwied Ekasari, Lusiana Ariyanti, Hadi Poerwono, Sukardiman*

*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*

*Presenting Author*
Email: sukardiman@ff.unair.ac.id, Phone: 081330458449/0818525342

ABSTRACT

**Objective:** The present study was carried out to evaluate the antidiabetic activity of diterpene lactone fraction of “Sambiloto” (*A. paniculata* Nees.)

**Methods:** Antidiabetic was induced experimentally by a single intraperitoneal administration of 3.8 mg/20 g body weight of Alloxan-monohydrat in each male mouse. Three days after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and were used in this study. Then the mice were randomly divided into five experimental groups of six mice each. First group as positive control (Glibenclamide 0.06 mg/20 g body weight). Second group as negative control (CMC-Na 0.5%). Samples were given by oral route at three dosage levels (7.4 mg, 14.9 mg, and 22.3 mg/20 g body weight). After treatment finished, blood sampling was done by sterilizing the tail with alcohol and then nipping the tail at the start of the experiment and this was repeated after 2nd, 4th, 6th, and 24th h.

**Conclusion:** Diterpene lactone fraction of “Sambiloto” (*A. paniculata* Nees.) has antidiabetic activity and the most effective dose was at 14.87 mg/20 g body weight of mice.

**Keywords:** *A. paniculata* Nees., Diterpene Lactone Fraction, Antidiabetic Activity, *in vivo*, Alloxan
Standardization Raw Material and Ethanolic Extract of Andrographidis Herba
(Andrographis paniculata Nees) from District of Bogor and Tawangmangu

Sukardiman*, Rakhmawati, Alvi Prabowo, Lusiana Arifianti

Department of Pharmacognosy and phytochemical, Faculty of Pharmacy, Airlangga University,
Surabaya, Indonesia

*Presenting Author
Email: sukardiman@ff.unair.ac.id, Phone: 081330458449/0818525342

ABSTRACT

Sambiloto (Andrographis paniculata Nees) has a potency to be developed in phytopharmaca industry, especially in this study focused on supporting anti- hyperglycemic research. This requires standardization which can affect both the raw material’s quality and extract which have been obtained based on the Indonesian Herbal Pharmacopeia method. Raw material for this study has been obtained from two traditional drug development institutions among others BALITRO from Bogor and BPTO from Tawangmangu.

Keywords: standardization, raw material, ethanolic extract, Andrographis paniculata Nees, Andrographis herbs
ABSTRACT

The synergy of antiinflammatory and immunosuppressant activity is very useful to ameliorate autoimmunity. The aim of this research was to investigate the anti-inflammatory and immunosuppressive activity of breadfruit, (Artocarpus alt挑选is [Park.] Fosberg) leaf, fruit and bark extract. The presence of flavonoids in crude extracts has been confirmed by the phytochemicals screening, quantification of total phenolic and flavonoid compound. Antiinflammatory activity testing was performed on Complete Freund’s adjuvant (CFA)-induced chronic inflammatory rats, which received extract treatment for 6 days. Immunomodulatory activity testing was performed on Swiss Albino mice. The relative weights of lymphoid organs, phagocytic index, delayed hypersensitivity type (DTH) response, and Haemagglutinin titer (HT) were studied in various animal groups. The result showed that the administration of breadfruit leaf extract at dose of 200 mg/kgBW caused a reduction of relative weight loss of liver and thymus significantly. Leaf extract at dose of 200 mg/kgBW and fruit extract at dose of 100 mg/kgBW elicited a significant decrease (p <0.05) in phagocytic index. The DTH response showed a significant decrease (p <0.05) in the fruit extract group and the breadfruit leaf at all doses. In the HT test, fruit extract and breadfruit leaves at dose of 200 mg/kgBW showed a suppressive effect on the immune response, while the other group showed a stimulatory effect. The results suggested that breadfruit leaf extract at dose of 200 mg/kgBW is more potent as an anti-inflammatory and immunosuppressant.

Keyword: Artocapus alt挑选is (Park.) Fosberg, antiinflammation, CFA-induced inflammation, immunomodulation, immunosuppression.
Cell Cycle Inhibition and Apoptosis Induction Activities of n-Hexane Fraction of *Cyperus rotundus* L. Rhizome

Masfria¹*, Urip Harahap², Denny Satria³

¹Department of Pharmaceutical Chemistry, ²Department of Pharmacology, ³Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Sumatera Utara

*Presenting author
Email: masfria@usu.ac.id, Phone: +62819641105

Abstract

**Background:** Breast cancer is one of the leading cause of death and the most common cancer type amongst women in the world after cervic cancer.

**Objective:** To evaluate the cell cycle inhibition and apoptosis induction activities on T47D cell lines of n-hexane fraction (nHF) of *Cyperus rotundus* L. rhizomes.

**Methods:** Ethanol extract was obtained by maceration method and was fractionated with n-hexane. Cytotoxic activity was examined with MTT assay, and cell cycle inhibition, apoptosis induction and cyclin D1 expression were assessed with flow cytometry method.

**Results:** Cytotoxic activity of nHF was found to have IC₅₀ of 71.69 ± 0.34 µg/mL for, nHF at concentration 35 µg/mL caused accumulation in G₀-G₁ and S phase accumulation (56.89% and 19.36%), increased early apoptosis (26.30%) and decreased expression of cyclin D1 (26.30%).

**Conclusions:** The results reveal that nHF of *Cyperus rotundus* L. rhizomes has cell cycle inhibition and apoptosis induction activities. Our further study is to isolation compounds which responsible for these activities.

**Keywords:** Cell cycle, Apoptosis, *Cyperus rotundus* L., rhizome, n-hexane.
In Vitro Antimalarial Activity of selected Indonesian plants grown in East Kalimantan

Hilkatul Ilmi1*, Diajeng Devita Koesoemawardhani2, Riza Rosita2, Sintya Putri Pratiwi2, Suciati3, Aty Widyawaruyanti1,3, Achmad Fuad Hafid1,3

1Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115; 2Graduate Student of Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60826; 3Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60826

*Presenting author
Email: tatun31@gmail.com

ABSTRACT

Background: Resistance to some of antimalarial drugs has been responsible for a recent increase in malaria-related mortality. Hence, new highly efficacious and affordable antimalarial agents are urgently needed. Natural product might become the strategy to find new antimalarial drugs, considering nature as a promising source of drugs.

Objective: This study was to determine the antimalarial activities of eight species of plants were collected from Balikpapan, East Kalimantan. The plants were Melicope glabra Blume and Luvunga scandens (Rutaceae), Artocarpus sericicarpus, Artocarpus anisophyllus, and Artocarpus dadah (Moraceae), Eusideroxylon zwageri, Alseodaphne elmeri, and Neolitsea cassiaefolia (Lauraceae).

Methods: Eight species of plants consist of leaves and stem bark were extracted with gradually using n-hexane, dichloromethane and methanol by ultrasonic assisted method. Antimalarial screening was tested using the histidine-rich protein II (HRP2) and microtest assay with concentration 100µg/ml. The IC50 value of active antimalarial extracts were determined with microtest assay.

Results: The results of HRP2 and microtest assay showed that 8 extracts out of 48 extracts have an antimalarial activity against Plasmodium falciparum. The Dichloromethane and n-hexane extracts of L. scandens leaves and n-hexane extracts of M. glabra stem bark were classified as a high activity of antimalarial with IC50 value of 0.44 µg/mL, 1.45 µg/mL and 1.35 µg/mL, respectively. The n-hexane extracts of L. scandens stem bark, dichloromethane extracts of A. anisophyllus leaves, n-hexane extracts of A. anisophyllus stem bark, n-hexane extracts of A. dadah leaves, and dichloromethane extracts of N. cassiaefolia leaves were classified as a promising activity of antimalarial with IC50 value of 6.21µg/mL, 7.68 µg/mL, 13.47 µg/mL, 10.41µg/mL, and 12.34 µg/mL, respectively. Phytochemical screening revealed the presence of terpenoid in all extracts.

Conclusion: Dichloromethane extracts of L. scandens (Rutaceae) leaves classified as a high activity of antimalarial substances. It was identified contain terpenoid and might be potential candidate of the antimalarial drug.

Keywords: In vitro, antimalarial activity, Indonesian plant extracts
Analgesic Activity of Ethanol Extract of *Rhapidophora pinnata* L.f Schott Leaves in Mice induced by Acetic Acid

Sumaiyah¹*, Masfria², and Aminah Dalimunthe³

1Department of Technology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara
2Department of Chemistry Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara
3Department of Pharmacology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara

Tri Dharma Street No.5, Medan, Indonesia, 20155

*Presenting author
Email: sumaiyah@usu.ac.id, Phone: +6285361323060

ABSTRACT

**Background:** *Rhapidophora pinnata*, Araceae Family, is suspected able to reduce pain.

**Objective:** This study aimed to determine the effect of *Rhapidophora pinnata* to reduce pain by acetic acid induced writhing response in mice.

**Methods:** This effect was examined by the acetic acid induced writhing response in mice. The animals were divided into five groups (n=5) and received ethanol extract of *Rhapidophora pinnata* at doses of 50, 100 and 200 mg/kg BW, CMC-na 0.5% as negative control and acetalos 200 mg/kg BW as positive control. This preparation was given orally 30 minutes before the given acetic acid 3% (w/v) as the pain inductor. Analgesic activity was measured by counting the percentage of writhing movements.

**Results:** The study showed that all of dose ethanol extract of *Rhapidophora pinnata* gave significant pain reduction in mice induced by acetic acid (p<0.05) compared to control group. The effective dose was shown by ethanol extract of *Rhapidophora pinnata* 50 mg/kg.

**Conclusion:** Ethanol extract of *Rhapidophora pinnata* can reduce pain in mice induced by acetic acid.

**Keywords:** analgesic, *Rhapidophora pinnata*, acetic acid, percentage of writhing movements
The Preparation Nanoparticles of Ethanol Extract of Pugun Tanoh (*Picria fel-terrae* Lour.) Leaves and Antibacterial Activity Test Against *Staphylococcus aureus* and *Escherichia coli*

Sumaiyah¹ and Nova Mustika¹

¹Department of Technology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155

*Presenting author
Email: sumaiyah@usu.ac.id, Phone: +6285361323060

ABSTRACT

**Background:** The manufacture of nanoparticles aims to improve bioavailability and modify drug delivery systems. *Picria fel-terrae* leaves contain flavonoids and tannins that are effective as antibacterials.

**Objective:** The research aimed to make nanoparticle ethanol extract of *Picria fel-terrae* leaves and to know the antibacterial activity of extract and nanoparticle extract on *Staphylococcus aureus* and *Escherichia coli*.

**Methods:** Nanoparticle ethanol extract of *Picria fel-terrae* leaves made with ionic gelation method. The formed nanoparticles are characterized by their size and morphology. Nanoparticle extracts tested antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

**Results:** The size of the extracted nanoparticles obtained is 464.22-653.19 nm. The morphology of extracted nanoparticles is aggregated with uneven surfaces. Effective barrier area with concentration 300-500 mg/ml are 12.81-14.48 mm for *Staphylococcus aureus* and 13.28-14.21 mm for *Escherichia coli*, while for extract nanoparticles with concentration 30-50 mg/ml are 12.98-14.40 mm for *Staphylococcus aureus* and 13.18-14.20 mm for *Escherichia coli*.

**Conclusion:** The ethanol extract of *Picria fel-terrae* leaves can be made into nanoparticles by ionic gelation method and extract nanoparticles provide better inhibitory power than extracts against *Staphylococcus aureus* and *Escherichia coli* bacteria.

**Keywords:** nanoparticles, ethanol extracts, *Picria fel-terrae* Lour. leaves, ionic gelation, antibacterial
Potency of Indonesian Marine Sponges as Acetylcholinesterase Inhibitor

Suciati¹*, Karma Rabgay² and Kornkanok Ingkaninan²

¹Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, East Java, Indonesia;
²Bioscreening Unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation of Chemistry, Naresuan University, Phitsanulok, Thailand

*Presenting author
Email: suciati@ff.unair.ac.id, Phone: 081231779793

ABSTRACT

Background: Alzheimer’s disease (AD) is a neurodegenerative disorder, which is the most common cause of dementia. The ageing population means that the number of people suffering with AD is expected to increase each year if there is no effective treatment found. One of the strategies for treatment of AD is the use of acetylcholinesterase (AChE) inhibitors as cholinergic deficiency is a common feature in the early stage of AD and in patients with mild cognitive impairment. The oceans with their unique and wide range of biodiversity, producing unusual metabolites, emerges as a good candidate for new therapeutic agents, including as acetylcholinesterase inhibitor.

Objective: To investigate the potency several marine sponges collected from Tabuhan Island, Banyuwangi, East Java as acetylcholinesterase inhibitor.

Methods: Marine sponges were freeze-dried and extracted with methanol. The anticholinesterase screening of the extracts were carried out according to the modified Ellman’s method. Every experiment was carried out in triplicate. Enzyme activity was calculated as a percentage of the velocity of the test sample compared with that of the non-treated control. Inhibitory activity was calculated by subtracting the percentage of enzyme activity from 100% enzyme activity. The IC₅₀ of inhibitory activity was analyzed using GraphPad Prism.

Results: From this preliminary screening of 15 sponge extracts, three sponge extracts, namely Callyspongia sp, Niphates olemda and Agelas nakamurai showed inhibition against AChE with % inhibition of 97.16, 91.89, and 85.66%, respectively. Inhibition of the three samples against butyrylcholinesterase (BuChE) were also investigated. The results showed that the extracts of Callyspongia sp, N. olemda and A. nakamurai inhibited BuChE at 93.65, 73.29 and 94.92%, respectively. Determination of the IC₅₀ of the samples were conducted against human AChE, the samples gave IC₅₀ of 14.69, 53.47, and 1.05 µg/mL.

Conclusion: Marine sponges from Tabuhan Island, namely Callyspongia sp, Niphates olemda and Agelas nakamurai showed potency as acetylcholinesterase inhibitor.

Keywords: sponges, acetylcholinesterase inhibitor, Alzheimer’s Disease, Callyspongia sp, Niphates olemda, Agelas nakamurai.
Antioxidant Activity and Hedonic Test on Gaharu Leaf Tea Based on Drying Method

Ridwanti Batubara¹,², T. Ismanelly Hanum³*, Iwan Risnasari¹,², Herawaty Ginting³ and Lusi Amelia Lubis¹

¹Fakultas Kehutanan, University of Sumatera Utara, Medan, 20155, North Sumatera, Indonesia
²Natural Resources Technology Based Center, Mangrove Group and Bio-Resources, University of Sumatera Utara, Medan.
³Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sumatera Utara Jl. Tridharma 5 Kampus USU Medan 20155

*Email: isma_nelly@usu.ac.id, Phone: +6285288691930

ABSTRACT

Background: Gaharu leaf tea can be developed as an alternative tea rich in antioxidants.

Objective: The aim of this study was to determine antioxidant activity and hedonic test to consumers in gaharu leaf tea (Aquilaria malaccensis Lamk) based on drying method.

Methods: The drying method of gaharu leaves was conducted using oven drying at 40°C, roasted and direct sunlight drying then the gaharu leaves water extract was tested for the antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method and hedonic test of gaharu leaf tea to consumers.

Results: The gaharu leaf water extract showed antioxidant activity with IC₅₀ values of 33.913 μg/ml; 25.544 μg/ml and 32.158 μg/ml with the oven drying at 40°C, roasted and direct sunlight drying, respectively. The hedonic test to consumers toward the drying result with the three methods were on a 3-4 scale with like moderately categories, however oven drying method were like extremely categories.

Conclusion: It is concluded that the roasted drying method of gaharu leaves has very strong antioxidant activity despite of the oven drying method are like extremely categories in hedonic test to consumers.

Keywords: Gaharu leaf tea, antioxidant, hedonic test, drying method
ABSTRACT

Since 2003 until May 2015, it is reported 840 influenza A (H5N1) cases in humans which caused 447 deaths worldwide. Due to the resistance of current antiviral drugs used to treat H5N1 infection, new antiviral is strongly needed. Many studies have reported antiviral activity of some bioactive compound from plants against several viruses. This raised a concern to utilized plants as a source of new antiviral agents. *Vitex trifolia* is one of Indonesia medicinal plants which have been reported to have several biological activities. In this study, the inhibitory activity of 96% ethanolic extract of *Vitex trifolia* leaves against H5N1 viruses was tested using two methods, hemagglutination (HA) assay and neuraminidase (NA) inhibition assay. Neuraminidase activity was conducted using MUNANA assay. In HA assay, it was found that at concentration 1000 µg/mL, the extract showed 60.67% of titer reduction. From the NA inhibition assay, it was found the IC$_{50}$ value was 82.62 µg/mL. Results concluded that 96% ethanolic extract of *Vitex trifolia* leaves could inhibit the H5N1 viruses with the mechanism as neuraminidase enzyme inhibitor. Thus, *Vitex trifolia* expected to be a source of new antiviral agents.

**Keywords:** *Vitex trifolia, H5N1, neuraminidase*
Standarization Parameters of Crude Drud and Ethanolic Extract of Cassia Spectabilis Leaves

Wiwied Ekasari1*, Tutik Sri Wahyuni1, Heny Arwaty2

1Departement of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.
2Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

*Corresponding author E-mail: wiwied-e@ff.unair.ac.id

ABSTRACT

The genus Cassia, comprising about 600 species widely distributed worldwide is well known for its diverse biological and pharmacological properties. Cassia spectabilis is widely grown as an ornamental plant in tropical and subtropical areas and has been commonly used in traditional medicine for many years. Ethanolic extract of the Cassia spectabilis leave have antimalarial activity both in vitro against Plasmodium falciparum and in vivo against P. berghei. The aims of this study are to know the specific and non-specific parameters of raw material quality standard of C. spectabilis leaves based on the requirement of Indonesian Herbal Pharmacopeia. The research methods used was experimental. Preparation of extract with maseration method used ethanol solvent. Based on the result of study, it can be concluded that the crude drug and ethanolic extract of C. spectabilis leaves meets the quality requirement to be used as traditional medicine raw material.

Keyword: Cassia spectabilis, Leaves, Standardization, Crude drug
PP83

In Vitro Cytotoxic Activity of Marine Sponges Against T47D and He La Human Cancer Cell Lines

Suciati1* and Lusiana Arifianti1

1Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia 60286.

*Presenting author
Email: suciati@ff.unair.ac.id, Phone: 081231779793

ABSTRACT

Background: Marine sponges have been known as the source of natural products. Various metabolites with potent bioactivities, such as anticancer, antifouling, antiviral, antimicrobial, and anti-inflammatory activities have been reported from this organism.

Objective: To investigate the anticancer potency of three marine sponges, namely Diacarnus debeauforti, Haliclona amboinensis, and Agelas cavernosa collected from Barrang Lompo Island, South Sulawesi, Indonesia.

Methods: Marine sponges were extracted with ethyl acetate. The anticancer screening of the extracts were done against T47D and He La cancer cell lines using MTT method.

Results: All three sponges gave anticancer activity against T47D cancer cell line, with the lowest IC50 of 18.2 µg/mL was given by extract of A. cavernosa. In the screening against He La cancer cell line, extract of D. debeauforti gave the highest potency with IC50 of 15.7 µg/mL.

Conclusion: Ethyl acetate extracts of D. debeauforti, H. amboinensis dan A. cavernosa showed potency as anticancer agents against T47D and He La cancer cell lines.

Keywords: sponges, Diacarnus, Haliclona, Agelas, anticancer
A Comparative Study of on the Antimicrobial Activity of Wild (Tualang) Honey and Artificial Honey against Methicillin-resistant Staphylococcus Aureus (MRSA), *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

May Florence Dela Cruz Bacayo\(^1\)*, Shazwan Fahmi Shafi\(^1\), Wong Charng Choon\(^1\), Santosh Fattepur\(^1\), Kiran Chanabasappa Nilugal\(^1\), Jiyauddin Khan\(^1\), Erwin Faller\(^1\), Fadli Asmani\(^1\), Eddy Yusuf\(^2\)

\(^1\)School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; \(^2\)International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: may_florence@msu.edu.my Phone: +603-55216488

**ABSTRACT**

**Background:** Antibiotics are becoming less effective due to emergence of these antibiotic-resistant bacteria, thus the discovery of alternative therapeutic agents is urgently needed. Honey is believed to have antimicrobial activity and used in traditional medicine as skin healing dressing for wound.

**Objectives:** To find an alternative method or substance as a substitute for the current antibiotic in the market and to compare the antimicrobial activity of the wild (*Tualang*) honey and artificial honey.

**Methods:** The 50 % (w/v) of honey are diluted using double dilution method and are tested for its antimicrobial activity using disc diffusion method with antibiotic Ampicillin for *S. pneumonia*, Gentamycin for *K. pneumonia* and Vancomycin for MRSA as positive control. The MIC and MBC for this honey also were determined in order to evaluate its antimicrobial activity.

**Results:** Based on the results obtained from the disk diffusion assay, it shows that wild (*Tualang*) honey has antimicrobial bacterial activity at concentration of 10 % (w/v) for MRSA and *S. pneumonia* while at 25 % (w/v) for *K. pneumonia*. Meanwhile it need 100% (w/v) concentration of artificial honey to shows its antimicrobial activity. Wild (*Tualang*) honey was recorded as the most potent honey against *S. pneumonia*, in which a dilution of 10% (w/v) was required to inhibit the growth and kill *S. pneumonia* colony at 20% (w/v). The concentration of 20% (w/v) was required to inhibit MRSA and 25% (w/v) to kill MRSA. Meanwhile, the highest concentration required to inhibit *K. pneumonia* at 50% (w/v) and no bactericidal effect were recorded.

**Conclusion:** The comparative study between Tualang honey and artificial honey gives a promising result that the Tualang honey has a highest antimicrobial activity against MRSA, *Klebsiella pneumonia*, *Streptococcus pneumonia* compared to the artificial honey.

**Keywords:** *Tualang Honey*, *Artificial honey*, *Antimicrobial activity*, *MRSA*, *Klebsiella pneumonia*, *Streptococcus pneumonia*
Evaluation of Anti-ulcer Effect of Papaya Juice in Combination with Cumin Seed in Ethanol Induced Peptic ulcers in Experimental Rats

Kiran Chanabasappa Nilugal¹, Visallani Veerapandian¹
Santosh Fattepur¹, Fadli Asmani¹ May Florence Dela Cruz Bacayo¹, Wong Charng Choon¹, Eddy Yusuf ²

¹School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia;
²International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: kirannilugal@gmail.com Phone: +603-55216488

ABSTRACT

Background: A disease which impairs the quality of life and associated with increased morbidity and mortality and a worldwide problem is known as peptic ulcer. Ulceration is resulting with the elevated acid secretion, and diminished mucosal resistance due to Helicobacter pylori infection, NSAID's, alcohol intake and tobacco smoking.

Objectives: This study was carried out to find out the anti-ulcer activity of papaya juice mixed with cumin seed extract in ethanol induced peptic ulcer in rats.

Method: Healthy adult female albino rats were used for this study. The rats were divided into five groups with six rats each. Omeprazole 20 mg/kg were used as a standard drug. Group 1: Control group, Group 2: Positive group, Group 3: Standard group which received Omeprazole, Group 4: Extract test group which received papaya juice (20 ml/kg) mixed with cumin seed with strength of (2.5 ml/kg), Group 5: Extract test group which receives papaya juice (20 ml/kg) mixed with cumin seed with the strength of (5 ml/kg). Ethanol will be given in dose of 1 ml per rat orally to Group 2, 3, 4 and 5 animal groups on the 8th day of experiment. The rats were sacrificed and stomachs were opened. Ulcer index and histological changes were studied.

Result: The results of the study exhibit that papaya juice in combination with cumin seed extract has significant anti-ulcer activity with p value <0.05 through statistical analysis and there is decrease in ulcer score comparable to omeprazole.

Conclusion: The extract group has had better ulcer protective compared to control indicating the plants possess significant anti-ulcer property in a dose dependent manner.

Keywords: Anti-ulcer effect, cumin seed, papaya, ulcer index.
Wound Healing Activity of *Ageratum Conyzoides* L. Leaves Extract

Mohamed Nizam Abdul Ghani*, Shakirah Suid, Jiyauddin Khan, Sri Budiasih, Samer Al-Dhalli, Mohammed Kaleemullah, Ibrahim Abdullah, Fadli Asmani, Eddy Yusuf

1School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; 2International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: m_nizam@msu.edu.my Phone: +603-55216488

ABSTRACT

**Background:** Wounds come in a few different varieties, regardless of what caused the wound; it constitutes a break in the skin and opens up the body to infection. Herbal plants are important in curing the wounds. A crude material isolated from the leaves of *A. conyzoides*, an herb widely used by traditional medicine men exhibit antibacterial activity as well as accelerate wound healing process. This is maybe due to its phytochemical constituents such as alkaloids, tannins, flavonoids and terpenoids.

**Objectives:** The aim of this research was to study and evaluate the potential of wound healing activities of *A. conyzoides* L. leaves extract.

**Method:** Ethanol extract of *A. conyzoides* L. was studied for the wound healing activities on cutaneous excision wounds in Wistar Albino rats. Wistar Albino rats are divided into four groups which consist of four rats in each group. The rats were placed individually in cages and all rats were experimentally wounded in the posterior back area. The simple ointment was applied topically to the wounds of Group 1 rats. Group 2 were treated with Betadine Ointment as a standard drug. Meanwhile, Group 3 and Group 4 were treated with 40% and 20% concentration of *A. conyzoides* L. ethanol extract respectively. The effects of extracts on the rate of wound healing were assessed.

**Result:** The studies on excision wound healing model (without infection) showed that there is almost complete healing (100% wound closure) on the 16th post wounding day with the ethanol extract. The result was obtained from the comparison between the different concentration of the extracts and with the control group (use Betadine and simple ointment). The best result shown by 40% of *A. Conyzoides* leaves extract followed by Betadine ointment, 20% extract, and last simple ointment.

**Conclusion:** The results of study showed that the extract ointments of *A. conyzoides* effectively stimulate wound contraction as compared to control group and standard group. These finding could justify the inclusion of this plant in the management of wound healing.

**Keywords:** Wound healing, *A. conyzoides* L., leaves extract etc.
The Study of Antibacterial of Asam Gelugor (Garcinia Atroviridis) Against the Methicillin-resistant Staphylococcus Aureus (MRSA), Streptococcus pneumoniae and Klebsiella pneumoniae

May Florence Dela Cruz Bacayo¹*, Nurul Shahira Sajali¹, Wong Charng Choon¹, Santosh Fattepur¹, Kiran Chanabasappa Nilugal¹, Jiyauddin Khan¹, Erwin Faller¹, Fadli Asmani¹, Eddy Yusuf²

¹School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; ²International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: may_florence@msu.edu.my Phone: +603-55216488

ABSTRACT

Background: Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. Some herbs have been reported to have antibacterial activity such as Garcinia Atroviridis.

Objectives: Asam Gelugor (Garcinia Atroviridis) extracts was evaluated for its antibacterial activity against microorganisms such MRSA, Streptococcus pneumoniae and Klebsiella pneumonia.

Method: Asam gelugor fruits were collected (600 g), the powder form was successively extracted with methanol (99.8%). The resultant solution was filtered and dried using a rotatory evaporator in a water bath at a temperature not exceeding 50°C. The antibacterial activity was determined using agar disc diffusion method for determination of the zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Zone of inhibition were compared with that of different standards like ampicillin, vancomycin and gentamicin for antibacterial activity.

Result: The test by using disc diffusion method shows highest inhibition against the K. pneumonia (15.33 ± 1.53) in 100% extract. Based on the result for MIC, inhibition is at 50 mg/ml until 0.05 mg/ml against MRSA, while the MIC is positive in all concentration of extract against K. pneumonia, but the MIC result is negative in all concentration of the extract against S. pneumonia. MBC result showed that there is bacterial growth in 500 mg/ml of extract against MRSA while no bacterial growth in extract against K. pneumonia, bacterial growth is positive in the extract against the S. pneumoniae.

Conclusion: Hence, these results show that Asam Gelugor (Garcinia Atroviridis) may serve as leads for the development of new antibiotic against this type of bacterial strains.

Keywords: Antibacterial Activity, Garcinia Atroviridis Extracts, Zone of Inhibition
Anti-hyperlipidemic of Methanolic Extract of Impatiens Balsamina L. in Hypercholesterolemic Induced Sprague Dawley Rats

Santosh Fattepur1*, Thurga Devi Balan1, Kiran Chanabasappa Nilugal1, May Florence Dela Cruz Bacayo1, Wong Chang Choon1, Mohamed Rasny Mohamed Razik1, Fadli Asmani1, Eddy Yusuf 2

1School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia;
2International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

*Presenting author
Email: dr_santosh@msu.edu.my Phone: +603-55216488

ABSTRACT

Background: The occurrence of hyperlipidemia is currently increasing at a remarkable rate throughout the world. Hyperlipidemia graded as one of the greatest risk factors that contributes to the prevalence and severity of life threatening coronary heart diseases. Medicinal plants and their products are safer than their synthetic counterparts, including those involved in the anti-hyperlipidemic drugs statins. Impatiens Balsamina (IB) is used medicinally for various ailments. No study was carried out on the anti-cholesterol activity of IB.

Objectives: To study anti-hyperlipidemic activity of methanolic extracts of IB (MEIB) in hypercholesterolemia induced Sprague Dawley rats.

Method: MEIB leaves were prepared using maceration method. Toxicity study was carried out using OECD guidelines. Hypercholesterolemia was induced by using 6% of lard oil, 2% of cheese and egg yolk. Two different doses 200 and 400 mg/kg of MEIB were used to study for anti-hyperlipidemic activity. Histopathological study was carried out in rats.

Result: No mortality was observed even up to 2 g/kg. Only 400 mg/kg of MEIB statistically decreased total cholesterol (P<0.05), LDL-cholesterol (P<0.05) and increase in HDL-cholesterol (P<0.05) when compared the control. Histopathology study showed 400 mg/kg administered groups have mild steatosis and no sign of inflammation in the liver cells as compared to control group.

Conclusion: MEIB could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia.

Keywords: Anti-cholesterol, Anti-hyperlipidemia, Impatiens Balsamina L., Simvastatin
Study on Biological Active Components of *Eurycoma longifolia*

Eddy Yusuf1*, Siti Shukriyah Shuhaimi2, Ryuichiro Suzuki3, Yoshiaki Shirataki3, Jiyauddin Khan2, Ibrahim Abdullah2, Fadli Asmani2, Nik Nur Shamiha Nik Dzulkefli2

1International Center for Halal Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; 2School of Pharmacy, Management and Science University, 40100 Shah Alam, Selangor, Malaysia; 3Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan

*Presenting author
Email: eddy@msu.edu.my Phone: +603-55216488

ABSTRACT

**Background:** Constant hyperglycemia in diabetic patient may lead to excess glycation and thus is believed to cause diabetic complications.

**Objectives:** *Eurycoma longifolia* (Simaroubaceae) is tested for inhibitory activity of advanced glycation end-products (AGEs) formation in vitro.

**Method:** Three concentration of methanolic extract were tested together with bovine serum albumin in anti-CML antibody. HRP-conjugated anti-mouse IgG antibodies were introduced, and sample were reacted with phenyldiaminedihydrochloride. Absorbance was read by using micro-ELISA and percentage of inhibition was calculated.

**Result:** The calculated percentage of AGEs formation inhibition by *E. longifolia* root are -3.62 % (0.1 mg/mL), 58.38 % (1 mg/mL) and 92.28 % (10 mg/mL) as compared to aminoguanidine 5.55 % (0.1 mg/mL), 39.32 % (1 mg/mL), 72.92 % (10 mg/mL) as referring to the concentration. Since the biological activity was tested on the whole methanolic extract, the activity is suggested to be due to synergistic activity of the extract.

**Conclusion:** New biological activity of *E. longifolia* methanolic extract which is inhibition of AGEs formation in vitro is seen. However, isolation of Fr.8-2, m/z: 381 does not lead to any compound isolated related to the plant.

**Keywords:** *Eurycoma longifolia*, Anti-glycation activity, % of inhibition, Tongkat Ali, AGEs formation inhibition in vitro.
Coumarins from the leaves of *Melicope denhamii*

Ratih Dewi Saputri¹, Tjitjik Srie Tjahjandarie¹, and Mulyadi Tanjung¹*

¹Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

*Presenting author
E-mail: mulyadi-t@fst.unair.ac.id

ABSTRACT

Three coumarins, xanthotoxine (1), bergaptene (2) and isopimpinellin (3) were isolated from the leaves of *Melicope denhamii*. Structures of compounds were elucidated using spectroscopic methods such as UV, IR, HRESIMS, 1D and 2D NMR. Compounds 1-3 were evaluated for their cytotoxicity against P-388 cells with MTT methods.

**Keywords:** Coumarins, *Melicope denhamii*, P-388 cell
Ethanol Extract Activity Test of 96% Sunflower Leaves (Helianthus annuus) towards Induction of Apoptosis, Cell Cycle, and p53 Cervical Cancer Cells HeLa Expression

Roihatul Mutiah1*, Jauza Ulfah1, M Firman Amrullah1, Abdul Hakim1, Ana Rahmawati2, Arief Suryadinata1

1Pharmacy Departement, Faculty of Medical and Health Sciences Maulana Malik Ibrahim State Islamic University of Malang, Indonesia
2Department of Medical Education, Faculty of Medical and Health Sciences Maulana Malik Ibrahim State Islamic University of Malang, Indonesia

ABSTRACT

Sunflower (Helianthus annuus) in addition to being used as an ornamental plant, it has also been proved to have anti-inflammatory pharmacological activities, anti-pain up to anti-malaria. The plant is known to contain flavonoids and sesquiterpen lactones substances so high so that alleged to have anti-cancer activity. The purpose of this research is to know the activity of ethanol Extracts of 96% sunflower towards the induction of apoptosis, cell cycle, and p53 Cervical Cancer Cells HeLa Expression.

Sunflower extracts are obtained by maceration extracts method using ultrasonic wave. Solvent that is used is 96% Ethanol. Activity Test is using IC50 value obtained by MTT method. The analysis of apoptotic induction and cell cycle using Flowcytometer and reading it with cell quest program. p53 expression measurements is using immunocytochemistry with p53 colorants antibodies.

The results show that values of IC50 sunflower leaf extract is 377.46 μg/mL; distribution of leaves extract with value concentration of IC50 increases the value of cell apoptosis of 7.17 % compared with controls (2.9 %) and increases the cell-lethal necrosis (90.44%). The sunflower extracts cause a decrease in the number of cells in the G0-G1 phase i.e. 33.98% and increases the number of cells in the S phase and G2-M each in consecutive 17.45% and 21.28% as well increasing the number of cells in the direction of sub G0-G1 (12.87%). The activity of the extract towards p53 showed a score of 0. The distribution of sunflower leaf extract improves cell apoptosis, influence cell cycle regulation and does not indicate an increased p53 expression.

Keywords: Sunflower Leaves extract, apoptosis, cell Cycle, p53, HeLa cervical Cancer Cells.
Antinociceptive and Antiinflammatory Activity of ST1 Derivated of Ethyl p-Methoxycinnamat in Mice Model Induced by Complete Freund’s Adjuvant

Juni Ekowati1, Junaedi Khotib2, Nike Dahlia1, Shigeru Sasaki3

1Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Dharmawangsa Dalam Street, Surabaya 60286, Indonesia
2Departement of Biomedic, Faculty of Pharmacy, Airlangga University, Dharmawangsa Dalam Street, Surabaya 60286, Indonesia
3Institute of Medicinal Chemistry, Hoshi University, Ebara 2-4-41, Shinagawa, Tokyo 1528501, Japan.

ABSTRACT

Ethyl p-methoxycinnamate isolated from Kaempferia galanga was used as starting material for synthesized of ST1-assisted microwave irradiation. The structure of ST1 (E)-3-(4-methoxyphenyl)-N-(phenylcarbamothioyl)acrylamide was analyzed by FTIR, 1H-NMR, 13C-NMR and HRMS spectroscopic methods. Antinociceptive activity in mice model (dosage 1.5 mg - 6mg; p.o) was tested in vivo using Complete Freund Adjuvant as inducer through intraplanar route compared with celecoxib 1.2mg. Our result showed that ST1 posses antinociceptive and anti-inflammatory activity which were indicated by an increase of resistance to heat stimulus and a decrease in plantar thickness in the legs of mice after CFA injection. In silico analysis predicted that its mechanism through inhibition of COX-2 enzyme.

Keywords: ethyl p-methoxycinnamate, antinociceptive, cyclooxygenase-2, CFA, (E)-3-(4-methoxyphenyl)-N-(phenylcarbamothioyl)acrylamide
Method validation for antibacterial activity test of aqueous extract of Rosella (Hibiscus sabdariffa L.) calyx against Escherichia coli ATCC 8739 using agar diffusion and microdilution

Isnaeni¹, Dyah Ayu Mitayani², Nuzul Wahyuning Diyah¹*

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286
²Undergraduate student, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

*Corresponding author
Email: nuzul wd@yahoo.com, Phone: +6281235188462

ABSTRACT
Background:
Aqueous extracts of Roselle calyx have antibacterial activities against Gram positive and negative bacteria including Escherichia coli. Agar diffusion and microdilution which have been used for antibacterial activity test are differs in observing the bacterial growth.

Objective:
This study attempts to compare the agar diffusion and micro dilution method for inhibitory activity of aqueous extract of Roselle calyx against Escherichia coli ATCC 8739 based on parameters among other, Limit of Detection (LOD), Limit of quantity (LOQ), linearity and precision.

Methods:
Sample preparation
The extract solution (10%) was made as infuse like and diluted until several concentrations, 2%, 4%, 6%, 8%, 10%, were obtained.

Agar diffusion method
Each solutions were assayed for their growth inhibition activity using agar diffusion with hole-reservoir. Escherichia coli ATCC 8739 was used as bacterial test and kanamycin was used as positive control. Minimum inhibition concentration (MIC) was observed as LOD, as well as LOQ, linearity and precision as the other validation parameters.

Micro dilution method
Each solutions of a serial dilution was added to Nutrient broth filled in 96-wells microtiter plate, each were inoculated with tested bacteria. After incubation at 37°C for 24 hours, 30 µL of 9% resazurine solution was added and plate was incubated again for 2 hours. Kanamycin was used as positive control. Microplate-reader was used for evaluating the bacterial growth. The observed parameters of validation that was similar to agar diffusion method.

Results:
It was found that The MIC or LOD of agar diffusion method was 0.5% . Correlation factor r and R² from linear equation, y = 11.131x+10.190, of the agar diffusion method was 0.998 and 0.996 respectively with repeatability precision of 7.9x10⁻³ while r and R² from linear equation, y = 2.022x+0.815, of the microdilution method was 0.939 and 0.882 respectively with repeatability precision of 6.32x10⁻².

Conclusion:
Based on the value of validation parameters, agar diffusion was better than microdilution method for inhibitory activity test of aqueous extract of Roselle calyx against Escherichia coli ATCC 8739.

Keywords: method validation, rosella, agar diffusion, microdilution.
Stability and inhibitory activity of red and purple Roselle aqueous extract against *Staphylococcus aureus* ATCC

Isnaeni\(^1\)*, Nana Rizki Widyasari\(^2\), Alfianita Rosyidah\(^2\), and Idha Kusumawati\(^1\)

\(^1\)Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286
\(^2\)Undergraduate student, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

\*Presenting author

Email: Isna.yudi @gmail.com, Phone: +6281331021303

**ABSTRACT**

**Background:** The use of aqueous extracts of Roselle calyx as a traditional beverage has been known in Indonesia. Two varieties that are frequently used are red and purple Roselle. Like other plant extracts, these two extracts are not stable at room temperature storage for three days. This research has been investigated the stability and the extract inhibitory activity against *Staphylococcus aureus* ATCC 25923 growth on storage temperatures of 8°C to ensure the safety of the product when it is consumed.

**Objective:** This study aims to establish the stability of aqueous extract of Roselle calyx var. red and purple after storage of one, two, three, four weeks, and two to three months. The measured stability parameters include physical appearance, spectra profile at 380-700 nm wavelength, chromatogram profile on Silica Gel GF\(_{254}\) TLC plate at light observation with 254 nm UV, inhibitory activity of the extract on *Staphylococcus aureus* ATCC 25923 growth.

**Methods:**

**Spectra profile**
Aqueous extract of Roselle (10 %) was made as an infusion and prepared in 7 series in a bottle for storage one, two, three, four weeks, two and three months. Observations on physical appearance and profile of spectra at 380-700 nm started in before and after storage for every time above. The spectra profiles were measured for diluted the extracts 1:25 and 1:50 of red and purple Roselle respectively to achieve appropriate results.

**Thin Layer Chromatography**
The TLC was performed by applying 6 µL of the samples solution on the Silica Gel F\(_{254}\) plate using formic acid: water: butanol (10:12:40) solution as an eluent. The chromatographic plate is dried, and the spot were observed by UV lamp at 254 and 366 nm. The whole profile of the chromatograms were measured and evaluated.

**Inhibitory activity**
A growth medium nutrient agar was seeded by suspension of *Escherichia coli* ATCC (Transmittance 25%) approximately 10\(^9\) colony forming units per ml. The seed medium was overlaid on the surface of base layer medium. Holes were created by perforating the agar medium used as samples reservoir, filled by the test solutions. After an overnight incubation at 37°C, diameter of growth inhibition zone of the test bacterial around that particularhole was observed and measured.

**Results:** The stability of the extract based on physical characteristics showed no change of color, flavor, and pH up to one-month storage with pH 2.58 ± 0.56. After 2 and 3 months storage, color changes occur, but spectral profiles in the 380-700 nm wavelength range are the same, giving maximum absorbance at 518 nm. The chromatogram profile of KLT silica Gel F\(_{254}\) using visible light shows the same overall profile after the three-week turnaround and begins to change after storage of four weeks to 3 months.

**Conclusion:** The Aqueous extract of red and purple Roselle are physically stable and based on inhibitory activity on the growth of *Staphylococcus aureus* ATCC 25923 after storage for three months at cold temperature (8°C).
Method validation for antibacterial activity test of ceiba honey against *Bacillus subtilis* ATCC 6633 using agar diffusion and turbidimetry

Nuzul Wahyuning Diyah¹, Laili Irfanah², Isnaeni¹*

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286
²Undergraduate student, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286

*Corresponding author
e-mail: isna.yudi@gmail.com, Phone: +6281331021303

**ABSTRACT**

**Background:** Honey was known to have bacteriostatic and good inhibiting action against the growth of Gram positive and negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*.

**Objective:** This study attempts to compare the agar diffusion and turbidimetric method for inhibitory activity of ceiba honey against *Bacillus subtilis* ATCC 6633 based on validation parameters among other, Limit of Detection (LOD), Limit of quantity (LOQ), linearity, and precision.

**Methods:**

*Sample preparation*

The honey solution (100%) was made in water solution and was diluted until several concentrations, including 60%, 70%, 80%, and 90%, were obtained.

*Agar diffusion method*

Each solutions were assayed for their growth inhibition activities on Nutrient agar inoculated with *Bacillus subtilis* ATCC 6633 at 25% transmittance. Test solutions and Kanamycin as positive control each were placed in hole-reservoir on the agar plate. Minimum inhibitory concentration (MIC) was observed as LOD, as well as LOQ, linearity, and precision which is selected as validation parameters.

*Turbidimetric method*

Each solutions of a serial dilution was added to Nutrient broth inoculated with 100 µL of test bacterial at 25% transmittance. After incubation at 37°C for 24 hours, the absorbance of the bacterial suspension was measured by spectrophotometer at 580 nm. Kanamycin solution (50 ppm) was used as positive control. Nutrient broth media was used as blank. Validation parameters of this method was similar to agar diffusion method.

**Results:** It was found that the MIC or LOD of agar diffusion and turbidimetric methods were 25% and 20% respectively. Correlation coefficient $r$ and $R^2$ from linear equation between log concentration and inhibition zone, $y = 13.023x + 15.296$, of the agar diffusion method was 0.983 and 0.966 respectively with repeatability precision of 0.032. Correlation coefficient $r$ and $R^2$ from linear equation between log concentration and % transmittance, $y = 192.527x + 52.360$, of the turbidimetric method was 0.982 and 0.964 respectively with repeatability precision of 0.022. The obtained correlation factor of both methods were indicate linearity.

**Conclusion:** Based on the value of validation parameters, agar diffusion method was similar to turbidimetry for inhibitory activity test of ceiba honey against *Bacillus subtilis* ATCC 6633.

**Keywords:** method validation, ceiba honey, agar diffusion, turbidimetry.
Molecular Surveillance of Dengue Virus in Bangkalan, Madura Island, Indonesia

Teguh Hari Sucipto1*, Tomohiro Kotaki2, Kris Cahyo Mulyatno1, Siti Churrotin1, Shuhai Ueda2, Puspa Wardhani1, Aryati1, Soegeng Soegijanto1, Masanori Kameoka2

1Indonesia–Japan Collaborative Research Center for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Airlangga University, Jl. Mulyorejo, Surabaya 60115, Indonesia
2Center for Infectious Diseases, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan

*E-mail: teguhharisucipto@staf.unair.ac.id

ABSTRACT

Dengue virus (DENV) infection is a major health issue in tropical and sub-tropical areas. Indonesia is one of the biggest dengue endemic countries in the world. In the present study, the molecular surveillance of DENV in Bangkalan, Madura Island, Indonesia was performed in order to obtain a clearer understanding of its dynamics in this country. A total of 359 blood samples from dengue-suspected patients were collected between 2012 and 2014. Serotyping was conducted using a multiplex Reverse Transcriptase-Polymerase Chain Reaction and a phylogenetic analysis of E gene sequences was performed using the Bayesian Markov chain Monte Carlo (MCMC) method. Seventeen out of 359 blood samples (4.7%) were positive for the isolation of DENV. Serotyping and the phylogenetic analysis revealed the predominance of DENV-1 genotype I (9/17; 52.9%), followed by DENV-2 Cosmopolitan type (7/17; 41.2%), and DENV-3 genotype I (1/17; 5.9%). DENV-4 was not isolated. The Madura Island isolates showed high nucleotide similarity to other Indonesian isolates, indicating frequent virus circulation in Indonesia. The results of the present study highlight the importance of continuous viral surveillance in dengue endemic areas in order to obtain a clearer understanding of the dynamics of DENV in Indonesia.

Keywords: Dengue virus, Bangkalan, Serotype, Phylogeny
Antiproliferative Effect of The Leaves of *Averrhoa carambola* Against T47D Cells

Lusiana Arifianti*1, Sukardiman1

1Department Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115

*Presenting author
Email: lusiana-a@ff.unair.ac.id, Phone: +6281931016555

ABSTRACT

**Background**: Drug development efforts to find new selective and safe drugs for cancer from natural resources are promising ones. Thenatural products are obtained in multiple or single compound. One of them is an ethanol extract of *Averrhoa carambola*.

**Objective**: The purpose of this study was to determine antiproliferative effect of ethanol extract of *Averrhoa carambola* leaves on the kinetics of T47D breast cancer cells proliferation.

**Methods**: Antiproliferative effect was tested by examining the doubling time effect of the extract against the proliferating cells by using MTT assay to determine its inhibition of the cell growth at-24, 48 and 72 hours incubation time and cells were counted within time courses.

**Results**: The growth profiles were then compared between treated and control cells by calculating the doubling times. leaves extract treated cells showed prolongation of the doubling times.

**Conclusion**: This result indicated that such extract possesses inhibitory effect or Antiproliferative effect against T47D cells

**Keywords**: *Averrhoa carambola* L., antiproliferative, doubling time, T47D cells
Honey as The Body Defense from *Toxoplasma Gondii* Infection

Heni Puspitasari¹*, R.A Diah Ratu², Lucia Tri Suwanti³a, Mufasirin⁴

¹Toxoplasma Study Group, Institute of Tropical Disease, Airlangga University, Surabaya
²Bachelor of Veterinary, The Faculty of Veterinary Medicine, Airlangga University, Surabaya
³Parasitology Departement, The Faculty of Veterinary Medicine, Airlangga University, Surabaya
⁴Coresponding author: tswant@gmail.com

*henipuspitasari486@gmail.com; HP. 085230157672

ABSTRACT

Toxoplasma gondii infection may cause liver derrangement and on the other hand honey has an ability in repairing damage of liver tissue due to disease process. This recent study has been made to find out the influence of honey to overcome the histopathological changes of liver caused by toxoplasma infection. Twenty-five male mice of 2-3 months old were divided into five groups id est P0, P1, P3 and P4. P0 as a control group was administered with 0,2 ml normal saline solution intraperitoneally, while the P1 with 0,08 ml dorsata honey orally. P2, P3 and P4 were infected with 1x10³ of *T. gondii* intraperitoneally. Before this treatment, P3 and P4 were given dorsata honey 0.08 ml and 0.12 ml respectively. Four days post infection all of mice were sacrificed and the liver were subjected for microscopic examination with H&E staining. Scoring method under Mordue was applied to the 3 histological changes, that were degeneration, necrosis and cellular infiltration. Than the result analyzed by Kruskal Wallis and followed by Mann Whitney test. There were so significantly different among the groups (p<0.05), that the conclusion was there a reduced damage of the liver and the more effective dose was 0.12 ml.

*Keywords: Toxoplasma gondii, Toxoplasmosis therapy, liver histopathology, honey*
Composition Ratio of Lactose and Corn Starch in Granule Capsule Formulation of 70% Ethanol Extract Justicia gendarussa leaves as Male Contraceptive

Bambang Prajogo EW\textsuperscript{1}, Esti Hendradi\textsuperscript{2}, and Pramudita Riwanti\textsuperscript{1}

\textsuperscript{1}Pharmacognosy Department, Faculty of Pharmacy, Airlangga University, Surabaya
\textsuperscript{2}Chemical Pharmacy Department, Faculty of Pharmacy, Airlangga University, Surabaya
e-mail: prajogo_ew@yahoo.com

ABSTRACT

The objective of this study was to make a good physical property of Justicia gendarussa granules with lactose and corn starch as filler. Optimizations were made into 3 formulas. The difference of each formula was in the ratio of corn starch and lactose. Formula 1 used ratio 3:7 for corn starch and lactose, Formula 2 used ratio 1:1 for lactose and corn starch, Formula 3 used ratio 7:3 for corn starch and lactose. Physical evaluation was held to evaluate and choose the best granule like flowability, fines content, angle of repose, moisture content, compressibility. The result for granules optimization, flowability formula 1 was $3.29 \pm 1.08 \text{ g/s}$, formula 2 was $6.04 \pm 1.80 \text{ g/s}$, formula 3 was $6.48 \pm 1.32 \text{ g/s}$. Angle of repose for F1, 2 and 3 were $30.54 \pm 1.14^\circ$, $29.98 \pm 0.34^\circ$ and $26.98 \pm 0.00^\circ$. Compressibility index were 12.00\%, 10.00\% and 11.99\%. Moisture content 1.82\%, 2.08\% and 2.75\%. Fines content were above 20\%. From the evaluation, F2 was selected as the best formula.

Keywords: Justicia gendarussa, lactose, corn starch
Comparison of Lipid Extraction Bligh and Dyer and Hara and Radin Methodson Research Quality Mackerel Oil (Rastrelliger sp.)

Yanu Andhiarto, Shelvyanti Khoirun Nissa

Prodi Farmasi Fakultas Kedokteran Universitas Hang Tuah

ABSTRACT

Mackerel (Rastrelliger sp.) is the resources of small pelagic fish which has high economic value as one of the fish that usually consumed by Indonesian people. Mackerel Oil (Rastrelliger sp.) is one of the result of marine fishery development product which have potential to develop in pharmaceuticals study, because Mackerel contains of high omega-3. In addition, high market demand for fish oil and the export low value of Indonesia's fish oil could be one of the opportunities to Indonesian fish oil producers to produces fish oil with good quality.

This study aims to analyze the differences of two types of lipid extraction methods, Bligh and Dyer, and Hara and Radin on concerning to the result and quality of mackerel oil (Rastrelliger sp.). This study used the experimental design of laboratory with the Completely Randomized Design (CRD) method. The sampling technique is used the probability of simple random sampling which used 10 kg of mackerel (Rastrelliger sp.).

The result of this study showed that the differences of Bligh and Dyer and Hara and Radin method gave the real impact on recovery yield and free fatty acid quality values of Bligh and Dyer head and Hara and Radin meats of mackerel oil (Rastrelliger sp.) because it has the significant value p<0.05, but didn’t gave the real impact on free fatty acid quality values of Bligh and Dyer meats and Hara and Radin head of mackerel oil (Rastrelliger sp.) because it has the significant value p>0.05. Mackerel oil which produced by Bligh and Dyer method has a yellow color, and smelled fishy and the mackerel oil produced by Hara and Radin method which is brownish black color and smelled fishy.

Keywords: mackerel oil (Rastrelliger sp.), Bligh and Dyer, Hara and Radin, free fatty acids, peroxide numbers, paraanisidine (p-anisidin).
Beneficial effect of supercritical carbon dioxide extracted (SC-CO$_2$) dabai (Canarium odontophyllum) pulp oil in hypercholesterolemia-induced SPF Sprague-Dawley rats.

Noor Atiqah Aizan Abdul Kadir*, Azrina Azlan, Faridah Abas, and Intan Safinar Ismail

1Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia;
2Research Centre for Excellence for Nutrition and Noncommunicable Disease, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia;
3Department of Food Sciences, Faculty of Food Science and Technology, 43400 UPM Serdang, Selangor, Malaysia;
4Department of Chemistry, Faculty of Science, 43400 UPM Serdang, Selangor, Malaysia.

*Presenting author
Email: atiqahaizan@yahoo.com Phone: +60 13 899 5409

ABSTRACT

Background: The demand for edible oil has been increasing with the growing of world population and consumers’ preference for vegetable oils over animal fats. Production of dabai (Canarium odontophyllum) pulp oil by supercritical carbon dioxide extraction is still relatively new in Malaysia and plausibe to be investigated.

Objective: This study aims to investigate the effect of supercritical carbon dioxide extracted (SC-CO$_2$) dabai pulp oil (DPO) in hypercholesterolemia induced rats

Methods: Male specific pathogen free (SPF) Sprague-Dawley rats were fed with high cholesterol diet for 4 weeks to induce hypercholesterolemia. The hypercholesterolemic rats were subsequently subdivided into positive control group (PG), low dose group (LG), high dose group (HG), and statin group (SG). The LG and HG groups were administered with 0.5% and 2% of SC-CO$_2$DPO, respectively for another 4 weeks. Changes in body weight and biochemistry profile were measured.

Results: Hypercholesterolemic rats showed elevation of body weight and significant increment in total cholesterol (TC) and LDL levels when compared with normal rats consumed normal basal diet (NG) (p<0.05). Paired-samples t-test revealed that LG group showed a significant reduction in TC, triglyceride and LDL levels (p<0.05) and 8.26% increment in HDL level. Meanwhile, reducing trends in TC, triglyceride and LDL levels were detected in HG group. No significant differences in AST and ALT levels were detected in LG and HG groups when compared with NG group.

Conclusion: These findings demonstrated that SC-CO$_2$DPO contained potential factors contributing to cholesterol lowering effect and may serve as specialty oils for health promotion and disease prevention.

Keywords: Canarium odontophyllum, dabai pulp oil, hypercholesterolemia, supercritical carbon dioxide extraction
Profile of Sterol and Triterpenes in the Callus Culture of *Solanum mammosum*

Silvy Juliana¹, Suciati¹* and Gunawan Indrayanto¹

¹Plant Biotechnology Research Group, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, East Java, Indonesia

*Presenting author
Email: suciati@ff.unair.ac.id, Phone: 081231779793

**ABSTRACT**

**Background:** Several sterols and triterpenes have been reported in several *Solanum* spp cell cultures. In our previous study, the callus culture of *Solanum mammosum* produced cholesterol, campesterol, stigmasterol, and β-sitosterol, whilst solasodine was not detected. After sub culturing for many years, the *S. mammosum* callus cultures developed into two types of forms, i.e., fines and globular compact structure (CGS).

**Objective:** To investigate the effect of formation of the compact globular structure of *S. mammosum* callus culture to the profile of sterol and triterpenes.

**Methods:** Morphological examination of the fines and CGS callus cultures were done microcopically. The Sterol and Triterpenes profile were made in GC-MS. Identification of the metabolites were undertaken by comparison of MS of sample to standard and to the data available in databases.

**Results:** Both forms of callus cultures (fines and CGS) produce the same sterol such as cholesterol, campesterol, stigmasterol, sitosterol, and isofucosterol. Both callus cultures also contain triterpenes: cycloartenol, lupeol, 24-methylene cycloartanol, and Serratenediol. However, betullinic acid only present in the CGS callus culture.

**Conclusion:** The formation of CGS callus culture of *S. mammosum* has an effect on the profile of sterol and triterpenes.

**Keywords:** *Solanum mammosum*, callus, compact globular structure, sterol, triterpenes.
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