Lampiran 8. Surat Undangan sebagai Invited Speaker

UMS
UNIVERSITI MALAYSIA SABAH
BIOTECHNOLOGY RESEARCH INSTITUTE
Universiti Malaysia Sabah,
UMS Road, 88400
Kota Kinabalu, Sabah, Malaysia.
Tel : (+6088-320 991)
Fax : (+6088-320 993)
Email : pejipb@ums.edu.my

23 June 2017

Kholis A. Audah, PhD
Vice Director of Research
Swiss German University
Edu Town BSD City
Tangerang 15339-INDONESIA

Dear Dr,

INVITATION TO BIOTECHNOLOGY RESEARCH INSTITUTE, UNIVERSITI MALAYSIA SABAH

I am very much pleased to invite you visit Biotechnology Research Institute, Universiti Malaysia Sabah (BRI-UMS), on **13-18 July 2017.** The visitation aims :

1. To have your talk as a a guest speaker in our Eminent Speaker Series on 14 July 2017, at 10.00, in BRI-UMS
2. To have further discussion on possible research and academic collaboration activities between our institutes.
3. To have courtesy visit to UMS facilities at Kota Kinabalu and Sandakan, Sabah.

It is an honor for us to have you in Sabah. Please feel free to contact us if you need any further information

Sincerely yours,

Dr Cahyo Budiman
Deputy Director for Research and Innovation
Biotechnology Research Institute
Universiti Malaysia Sabah

Lampiran 9. Surat undangan presentasi oral

THE 3RD INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCES & BIOTECHONOLOGY 2017
DEPARTEMEN BIOLOGI
FAKULTAS MATEMATIKA DAN ILMUPENGETAHUAN ALAM - UNIVERSITAS SUMATERA UTARA
JL. BIOTEKNOLOGI NO. 1, MEDAN 20155
Contact person : It Jamilah (081387839793), Nunuk Priyani (08196000108)

No. : 11./FMIPA/ICBSB/VI./2017
Object : Seminar Invitation
Attachment : 1 (one) copy

Dear Participants,

The committee of 3rd International Conference on Biological Sciences and Biotechnology (ICBSB) is pleased to invite you for your participation in the seminar as **Oral Presenter** that will be held on:

Date / Time	:	August 23rd, 2017 / 08.00 a.m. (Registration starts at 07.30 a.m.)
Location	:	Hotel Arya Duta Medan Jl. Kapten Maulana Lubis No.8, Petisah Tengah Medan Petisah, Kota Medan, Sumatera Utara 20112

As a reminder, you are required to pay for the registration fee to Account Name: **ICBSB 2017**; Account Number: **0550408295** (Bank BNI) before August 15th, 2017. We would also like to inform you that the full paper of your article is still under review for the proceedings. Please check our websites regularly regarding the review process.
In addition, we need you to provide the **full name with title** of each presenters under your registered name to be printed in the certificate.
Thank you for your participation and we look forward to seeing you in Medan.

Chairman of ICBSB 2017,

Prof. Dr. Dwi Suryanto, M.Sc

Secretariat: Department of Biology, University of Sumatera Utara

Lampiran 10. Surat undangan sebagai penulis bab buku

UMS
UNIVERSITI MALAYSIA SABAH
INSTITUT PENYELIDIKAN BIOTEKNOLOGI
Jalan UMS,
88400 Kota Kinabalu, Sabah Malaysia
Tel : +6088-320000 / 320991
Faks : +6088-320993
Email : pejipb@ums.edu.my

Date: 06 September 2017

TO WHOMSOEVER IT MAY CONCERN

To
Kholis Abdurachman Audah, PhD
Department of Biomedical Engineering, Swiss German University, Prominence Tower, West Sutera Road, South Tangerang 15143, Banten Province, Indonesia

Dear Colleague,

I am inviting the chapters from eminent scientists working in this area at different laboratories including you. The main aim of this book publication is to compile the information on cutting edge researches on the emerging issues in-banner of book title is "**Nanotechnology: Applications in Energy, Drug and Food"** to be published by **Springer Publisher (Cham, Switzerland)**. We, therefore, invite you to contribute a chapter on title "**Drug Discovery: A Biodiversity Perspective**". We will send you further necessary guidelines of the book.

Yours sincerely

Editor

Shafiquzzaman Siddiquee

.....

(Shafiquzzaman Siddiquee)
Associate Professor
Biotechnology Research Institute
Universiti Malaysia Sabah
(Cellphone: +60149294481)

BERTEKAD CEMERLANG

www.ums.edu.m

Lampiran 11. Surat undangan sebagai peserta konferensi (penyaji poster)

INTERNATIONAL BIOTECHNOLOGY CONFERENCE ON ESTATE CROPS (IBCEC) 2017

IndonesianResearch Institute for Biotechnology and Bioindustry
Jl. Taman Kencana No.1, Bogor 16128- Indonesia
Phone: (0251) 8324048, 8327449 Fax: (0251) 8328516 Email: info@ibcec.net

LETTER OF INVITATION

Bogor, October 11th 2017

Dear Mr Julkipli

The Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) will host the **International Biotechnology Conference on Estate Crops (IBCEC) 2017** on October 18th – 20th 2017. This conference is in conjunction with the World Plantation Conference and Exhibition 2017 (WPLACE-2017).

This is to remind and invite you to attend IBCEC 2017 at the Grand Sahid Jaya Hotel, Jalan Jenderal Sudirman Kav. 86, Tanah Abang, Jakarta.

We also inform you that your abstract has been accepted by Scientific Committee of IBCEC-2017 under Microbe and Bioprocess Category with poster code C-09. For your information, the selected full paper will be published in IOP Proceeding : Environmental and Earth Science. We expect to accept the full-poster paper no later than 1 month after the conference. A peer-review process will be carried out for your paper. If accepted, the publication fee (1 million IDR) will be subjected to authors.

Details of the conference can be found on www.ibcec.net. Please do not hesitate to contact us if you have any further question.

Best regards,
Organizing Committee

Dr. Asmini Budiani

Lampiran 12. Poster konferensi

Pusat Studi Biofarmaka Tropika LPPM-IPB

Introduction of bioprospecting opportunities for Indonesian mangrove species

Julkipli[1], RR Batubara[2], GE Jogia[1], I Batubara[2], KA Audah[1*], KN Nunuk[2], H Sutanto[1]

[1]Department of Biomedical Engineering, Swiss German University, The Prominence Tower, Jalan Jalur Sutera Barat Kav 15, Alam Sutera, Kota Tangerang 15143, Banten, Indonesia, [2]Pusat Studi Biofarmaka IPB (LPPM IPB), Jl. Sempur Kidul No.69, Sempur, Kota Bogor, Jawa Barat 16129, Indonesia

*E-mail: kholis.audah@sgu.ac.id

Abstract. Indonesia is one of the world's most biodiverse countries. This study will be only focused on the Indonesian mangrove forests biodiversity. There are about three million hectares of mangrove forests that grew along the 95,000 kilometers of Indonesian coastline. Mangrove forests have ecology, social, economic and medicinal value that have been used by people who live along coastal area for centuries. Many studies shown that mangrove extracts contain many bioactive compounds that have the medicinal potential for a variety of diseases. However, mangrove plants extracts are yet to be commercially formulated as modern medicines. Although Indonesia is home to one of the largest biodiversity, the interest of pharmaceutical industries in the development of herbal medicine as drugs is not as promising as those from synthetics. One of the causes of this phenomena is the low interest of synthesizing bulks of natural products. In addition, that there has not been adequate facilities which can provide optimization of the herbal materials. The aim of this article is to give a rational approach for design a bioprospecting program as an initiation on the primary screening of novel drugs from Indonesian mangrove species.

Introduction

- Indonesian mangrove biodiversity represents approximately 22.6% of the total mangrove ecosystems in the world (Giesen et al., 2006; Giri et al., 2011).
- A number of mangrove's secondary metabolites have significant pharmacological properties that have been used traditionally for treatment of number diseases (Bandaranayake, 2002).
- Mangrove plants have numerous bioactive compounds should be suitable for biopropecting program.
- The aim of this article is to give a rational approach for design a bioprospecting program as an initiation on the primary screening of novel drugs from Indonesian mangrove species. Also, as a Following up the research of establishment extract library of Indonesian mangrove which is initiated by Audah et al. (2016).

Research methodology

Results and Discussions

- The extraction was conducted by remaceration with four different solvent (hexane, ethyl acetate, ethanol and water) and yielded 64 extracts.
- most extract are effective against gram positive bacteria and have potential to be anticancer agent. Mangrove plants that used in the research are 8 species out of more than 20 species, so there is more bioactive compound that has not been explored since different species might contain different bioactive compound.
- The crude extracts that are shown significant activity will be further fractionated and isolated to obtain a single compound
- The single compound can be optimized to achieve safety and efficacy according to established medical and standard requirements. Preclinical testing of mammalian objects from mice, rabbits, or even primates will be performed to ensure the desired safety and efficacy.
- Access to genetic resources should be undertaken taking into consideration the equitable sharing of benefits from the resulting product To be able to make genetic resources as the backbone of socioeconomic development, human resource development, science and technology capability, market analysis, sustainable capital, and strategic plan should be developed (Senthilkumar et al., 2012; Juan, 2017).

Conclusions

- Mangrove plants are potential for bioprospecting program
- Bioprospecting should be based on sustainable use of biodiversity.

Acknowledgments

The author would like to express the deepest gratitude to thank
- SGU (Swiss German University), ARCS Director and SGU ARCS staff
- lecturers staff of FK UNILA , DKKP staff and all parties who have helped the author's research activities either directly or indirectly

References

1. Giesen et al. (2006). *Mangrove guide book for Southeast Asia*. FAO and Wetlands International, Thailand: Dharmasarn Co., Ltd
2. Giri et al. (2011). Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography*, 20(1), 154–159.
3. Bandaranayake, W.M. (2002). Bioactive Compounds and Chemicals Constituents of Mangrove Plants. *Wetland Ecology and Management 2002*, 10(6), 421-452.
4. Audah K.A. (2016). Development of Extract Library from Indonesian Biodiversity Towards National Independency in Drug Discovery. Prosiding Seminar Nasional Kimia-Lombok 2016. No. B002, 11-19
5. Senthilkumar et al., (2012). Bioprospecting the Renewable Forest Resources: An Overview. *Current Biotica*, 5 (4), 522–540.
6. Juan B. 2017. Bioprospecting and Drug Development, Parameters for a Rational Search and Validation of Biodiversity. J Microb Biochem Technol 2017, 9:1. DOI: 10.4172/1948-5948.1000e128

Lampiran 13. Absensi kegiatan FGD ”Pembentukan Konsorsium Extract Library Indonesia“ LIPI

SGU
SWISS GERMAN UNIVERSITY

FOCUS GROUP DISCUSSION
ESTABLISHMENT OF INDONESIAN EXTRACT LIBRARY CONSORTIUM: OPPORTUNITIES AND CHALLENGES
The Prominence Tower, 27 July 2017

NO	NAME	INSTITUTION	ADDRESS			REMARKS	SIGNATURE
			HOME /OFFICE	EMAIL	HANDPHONE		
1	Dr. Eng Agus Haryono	Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia					
2	Prof. Hanafi Muhammad	LIPI					
3	Dr. Irmanida Batubara	Institut Pertanian Bogor					
4	Lany Marliany S.Si., Apt	PT Indofarma					
5	Kholis A. Audah, Ph.D	Swiss German University					
6	Dr. Heru Susanto	LIPI					
7	Evi K	FK unila					
8							
9	Eti Rohaeti	Kimia - IPB					
10	Mohamad Rafi	Trop Biol - IPB					
11	Henny Saraswati	Univ. Esa Unggul					
12	HE						
13	Dra Farmila	SGU					
14	Vera	SGU					
15	Eny ARCS	SGU					
16	Cindy ARCS	SGU					
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Lampiran 14. Dokumentasi kegiatan FGD “Pembentukan Konsorsium Extract Library Indonesia“

Lampiran 15. Persediaan sampel

No.	Nama Latin	Bagian	Bobot basah (g)	Bobot kering (g)	Susut (%)	Jumlah digunakan (g)
1.	*R. apiculata*	Daun	2000	440	78	50.01
						50.03
						50.01
2.		Batang	4000	2130	47	50.10
						50.02
						50.06
3.		Akar	2000	740	62	50.01
						50.01
						50.05
4.	*B. gymnorrhiza*	Daun	1500	560	63	50.02
						50.03
						50.02
5.		Batang	4100	1750	51	50.00
						50.00
						50.01
6.		Akar	2150	860	60	50.01
						50.01
						50.00
7.	*R. mucronata*	Daun	2800	820	71	50.01
						50.01
						50.01
8.		Batang	1100	580	47	50.02
						50.02
						50.02
9.		Akar	900	360	60	50.01
						50.01
						50.01
10.	*T. populnea*	Daun	650	190	71	50.01
						50.01
						50.00
11.		Buah	750	140	70	50.01
						50.01
11.	*A. marina*	Daun	1700	400	77	50.01
						50.01
						50.01
12.		Akar	1550	450	71	50.02
						50.04
						50.02
13.	*X. granatum*	Daun	1050	210	80	50.01
						50.01
						50.01
14.	*C. tagal*	Daun	1100	200	82	50.01
						50.01
						50.05
15.	*S. caseolaris*	Daun	1300	210	84	50.00
						50.01
						50.01

Lampiran 16. Sertifikat sebagai Oral Presenter, Best Oral Presenter, dan Participant.

Lampiran 17. Notification of Abstract Acceptance to Bromo Conference Symposium

14th May 2018

Kholis Abdurachim Audah
Swiss German University
Prominence Tower Alam Sutera
Tangerang, Indonesia

Re: Notification of Abstract Acceptance to Bromo Conference Symposium on Natural Products and Biodiversity on 11 – 12 July 2018, Surabaya, Indonesia

Dear Kholis Abdurachim Audah,

We are very pleased to inform you that your abstract entitled, **"Antibacterial Screening of Mangrove Extract Library: Accelerating Drug Discovery from Indonesian Biodiversity" (Abstract No. 196)** has been accepted for **oral_presenter** at Bromo Conference Symposium on Natural Products and Biodiversity scheduled on 11 – 12 July 2018 in Surabaya, Indonesia. The exact time and room of your presentation session will be specified on the Bromo Conference website: http://ff.unair.ac.id/conferences/bromo2018/ at the beginning of June, 2018.

Please note that individual requests for specific presentation dates and/or times cannot be addressed. Oral presentations cannot exceed 10 min (including disscussion). The details of oral presentation guideline is available on the conference website.

It is a condition of abstract acceptance that you or a nominated presenting co-author completes the registration and payment process. To register to attend the conference, please follow the link: http://ff.unair.ac.id/conferences/bromo2018/reg1

Should the addressee above not be the nominated presenter, please inform us the name and email address of the presenter immediately to: scientific-bromo2018@ff.unair.ac.id.

Again, congratulations on the acceptance of your abstract. If you are interest to publish your full paper to our proceeding or journal of Natural Product communication please submit your full paper to scientific-bromo2018@ff.unair.ac.id. On behalf of the Scientific Program Committee, we look forward to your full participation in the Bromo Conference in Surabaya.

Yours Sincerely,

Prof. Bambang Prajogo EW
Chairman of the Organizing Committee
Faculty of Pharmacy, Universitas Airlangga
Dharmawangsa Dalam, Surabaya, 60286, INDONESIA
E-mail: bromo2018@ff.unair.ac.id
Website: http://ff.unair.ac.id/conferences/bromo2018/

Lampiran 18. Full Paper Submission Notification to Bromo Conference Symposium

July 2018

Kholis Abdurachim Audah
Swiss German University
Prominence Tower Alam Sutera
Tangerang
Indonesia

Re: Full Paper Submission Notification to Bromo Conference Symposium on Natural Products and Biodiversity on 11 – 12 July 2018, Surabaya, Indonesia

Dear Kholis Abdurachim Audah,

Thank you for your manuscript submission entitled, **"Antibacterial Screening of Mangrove Extract Library: Accelerating Drug Discovery from Indonesian Biodiversity"** to the committee of Bromo Conference 2018. Soon, we will process the manuscript for peer review and send the review result back to you.

Please note that the payment should be completed soon after submission through our treasurer account as follows:

Bank Negara Indonesia (BNI)
Name: Idha Kusumawati.
Account no. 0604913380

Should you have any further question, please contact us at scientificbromo2018@ff.unair.ac.id.

Best regards,

Prof. Bambang Prajogo
Chairman of the Organizing Committee

Faculty of Pharmacy, Universitas Airlangga
Dharmawangsa Dalam, Surabaya, 60286, INDONESIA
E-mail: bromo2018@ff.unair.ac.id
Website: http://ff.unair.ac.id/conferences/bromo2018

Bromo Conference
Symposium on Natural Product and Biodiversity
Universitas Airlangga, Surabaya, 11th-12th July 2018

Lampiran 19. Letter of Invitation sebagai pembicara pada SYMOMATH 2018

SYMPOSIUM ON BIOMATHEMATICS (SYMOMATH) 2018
UNIVERSITAS INDONESIA
FACULTY OF MATHEMATICS AND NATURAL SCIENCE
DEPARTMENT OF MATHEMATICS
Building D the 2nd Floor, Kampus UI Depok, 16424. Phone. +621 7863439, +621 7862719.
URL: www.math.ui.ac.id/symomath2018/

LETTER OF INVITATION

July 1, 2018

Dr. Kholis A. Audah
Swiss German University,
Indonesia

Dear Dr. Kholis A. Audah,

On behalf of the Organizing Committee, we are pleased to invite you to participate the 2018 Symposium on Biomathematics (SYMOMATH 2018) as an invited speaker to be held at the Universitas Indonesia in Depok, Indonesia, 31 August – 2 September, 2018.

This letter is provided as an official invitation to enable you to attend SYMOMATH 2018.

Any inquiries relating to this Letter of Invitation should be directed to me at aldiladipo@sci.ui.ac.id

We look forward to welcoming you to Indonesia!

Sincerely,

Dipo Aldila
General Chair SYMOMATH 2018
Department of Mathematics
Universitas Indonesia
Indonesia
Email: aldiladipo@sci.ui.ac.id

Lampiran 20. Notification of Abstract Acceptance to IST 4 Symposium

IST4

THE 4TH INTERNATIONAL SYMPOSIUM ON TEMULAWAK AND POTENTIAL PLANTS FOR JAMU

28 August 2018 | Hotel Santika, Bogor | Bogor, Indonesia

"from Temulawak and Potential Plants for Jamu to their Modern Drugs and Cosmetics Advancements"

July 7, 2018

LETTER OF ACCEPTANCE

Dear Kholis Abdurachim Audah, Ph.D

Thank you for your interest to participate in the 4th International Symposium on Temulawak and Potential Plants for Jamu (IST4), which would be held on August 28, 2018 at Hotel Santika Bogor, West Java, Indonesia

On behalf of the IST4 scientific committee, we are pleased to inform you that your abstract with the title **Methods Development for Mangrove Extraction towards Standardization of Indonesian Extract Library** has been accepted as **oral presentation** in the IST4. The committee will not bear any airfare, accommodation and not provide any financial reward for your work during your visit in the symposium.

We are looking forward to seeing you in the IST4 and share your experiences and expertise with other participants.

Sincerely yours,

Organizing Committee of IST4
Chairman,

IST4

Dr. Eng. Wisnu Ananta Kusuma, MT

Lampiran 21. Surat Undangan Rapat dengan P2 Informatika dan SGU

LEMBAGA ILMU PENGETAHUAN INDONESIA
(INDONESIAN INSTITUTE OF SCIENCES)
PUSAT PENELITIAN KIMIA
Kawasan PUSPIPTEK Serpong, Tangerang Selatan 15314
Telp. (+62 21) 7560929, Faks (+62 21) 7560549
website : http://kimia.lipi.go.id, email : rochem@mail.lipi.go.id

Serpong, 18 April 2018

Nomor : B- 686 /IPT.2/UM.01/IV/2018
Lampiran : 1 (satu) halaman
Perihal : Undangan Rapat dengan P2 Informatika & SGU

Kepada Yth
Bapak/Ibu
(Daftar nama terlampir)
Di Tempat

Dengan hormat,

Sehubungan dengan agenda penandatanganan kerjasama pembentukan Konsorsium Indonesia untuk *Extract Library* Bahan Alam, maka kami mengundang Bapak/Ibu untuk hadir dalam acara yang akan dilaksanakan pada:

Hari/Tanggal : Jum'at, 20 April 2018
Waktu : 09.00 – selesai
Tempat : Ruang Rapat Lt. 1 P2 Kimia LIPI
Agenda : Pembahasan kerjasama dan rencana kegiatan konsorsium

Demikian undangan ini kami sampaikan, atas perhatian dan kerjasama Bapak/Ibu kami ucapkan terima kasih.

Kepala

Dr. Eng. Agus Haryono

NPC Natural Product Communications

20XX
Vol. XX
No. XX

Antibacterial Screening of Mangrove Extract Library: Accelerating Drug Discovery from Indonesian Biodiversity

Razethy Batubara[a], Julkipli Amsyir[a], Irmanida Batubara[b], and Kholis Abdurachim Audah[a*]

[a]*Department of Biomedical Engineering, Faculty of Life Sciences and Technology, Swiss German University, Tangerang 15143, Indonesia*
[b]*Biopharmaca Tropica Research Center, Bogor Agricultural University, Bogor 16128, Indonesia*

kholis.audah@sgu.ac.id

Received: January XX, 2018; Accepted: XX, 2018

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. Four types of solvents with different polarity were used producing 64 extracts. Disk diffusion method was used for antibacterial screening using five bacterial strains. There were 37 extracts showed antibacterial potential with the lowest and the highest recorded inhibition index were 0.0283 and 1.8983, respectively. The highest inhibition index was recorded for ethyl acetate extract of root of *Bruguiera gymnorrhiza* (77 Ea) screened against *Escherichia coli*. The second highest inhibition index was 0.7867 recorded for leaf of water extract of *Avicennia marina* (84 A) screened against *Staphylococcus aureus.* Phytochemical analysis of the extracts were also evaluated. The majority of samples showed saponin and tannin in considerable amount. This supported the data that mangrove extracts were potential as antibacterial agents.

Keywords: *Antimicrobial, drug discovery, drug resistant, extract library, Indonesian biodiversity*.

Drug discovery is a lengthy and expensive process. On the other hand, different types of diseases or drug resistant pathogens are increasing in numbers from time to time. The World Health Organization once reported that as many as 30 new diseases could emerge in 20 years period of time [1]. Therefore, finding alternatives for drug sources is urgently required. Drug discovery through screening process utilizing natural products can become a solution of the slow and expensive drug discovery process using conventional way.

Indonesia is well known as one of the richest country in the world in biodiversity [2]. The country possesses approximately 14000 islands, located between Indian and Pacific Oceans. According to Fauna and Flora International (FFI), Indonesia is home of approximately 11% or more than 30000 of the world's flowering plants and other biota both in land and marine with significant figures [3].

One of potential plants as medicinal sources and widely spread along Indonesian coastline is Mangrove. Mangrove and mangrove associates are very potential plants as medicinal sources [4]. Along roughly 90000 kilometer coastline, Indonesia is home of about 20 families with hundreds species of mangroves and their associates. Indonesia has the largest mangrove forest or about 23% of total world mangrove forests [5].

The aim of this study was to evaluate the potential of mangrove extracts (extracts library) as antibacterial agents. Since long time ago different parts of mangrove trees either roots, branches, leaves, flowers and the fruits had been utilized as food and medicinal sources.

Previous qualitative phytochemical studies showed that leaf extract of Rhizophora stylosa and Avicenna marina contain flavonoid, terpenoid, alkaloid, flavonoid and glycosidic phenolic [6]. Bioactivity of mangrove extracts against other types of diseases had also been reported.

Water content of simplisia (Tabel 1) showed that 15 out of 16 samples were kept below 10% which is a suitable percentage for simplisia analysis according to Indonesian Herbal Pharmacopeia [7]. Only one sample with sample code 83 which was originated from fruit of *Thespesia populnea* showed a slightly higher water content than 10% (10.06%).

Table 1: Water content of simplisia*.

Samples code	Water content (%)	Samples code	Water content (%)
72	8.60	81	7.56
73	2.30	82	7.54
74	7.36	83	10.06
75	5.34	84	9.43
76	3.57	85	8.55
77	8.23	86	8.98
79	4.78	87	9.45
80	4.85	88	7.23

*Water content of simplisia should be below 10% suitable for analysis.

The yield of extracts of the 16 samples were between 0.14% (highlighted green) to 26.23% (highlighted yellow) that belonged to *n*-hexane extract of root of *Rhizophora mucronata* and water extract

of leaf of *Avicennia marina*, respectively as shown in Table 2. The data clearly showed that root extract using non polar solvent (*n*-hexane) resulted in lower yield percentage compared to more polar solvents (samples 74, 77, 81 and 85). This indicated that root sample contains less nonpolar constituents compared to other parts of plants.

Table 2: Yield of extracts (%)*

Samples code	Yield	Samples code	Yield	Samples code	Yield	Samples code	Yield
72H	1.28	76H	0.26	81H	0.14	85H	0.5
72Ea	3.22	76Ea	0.27	81Ea	0.15	85Ea	0.89
72Et	6.54	76Et	2.3	81Et	3.6	85Et	1.98
72A	16.2	76A	5.45	81A	2.53	85A	10.31
73H	0.42	77H	0.29	82H	2.46	86H	0.76
73Ea	0.43	77Ea	0.32	82Ea	2.73	86Ea	1.79
73Et	3.3	77Et	8.95	82Et	3.55	86Et	6.94
73A	1.84	77A	4.14	82A	15.1	86A	21.18
74H	0.25	79H	1.04	83H	3.08	87H	2.41
74Ea	0.25	79Ea	2.36	83Ea	3.64	87Ea	2.27
74Et	11.92	79Et	2.48	83Et	2.99	87Et	10.86
74A	6.31	79A	13.26	83A	13.41	87A	19.58
75H	2.6	80H	0.26	84H	1.7	88H	1.52
75Ea	1.34	80Ea	0.23	84Ea	1.4	88Ea	1.43
75Et	7.09	80Et	4.88	84Et	7.42	88Et	9.46
75A	20.32	80A	3.72	84A	26.23	88A	7.06

*Solvents abbreviation: H = *n*-hexane, Ea = ethyl acetate, Et = ethanol, A = water.

Antibacterial screening of mangrove extracts in this study were targeted against gram positive bacteria represented by *Staphylococcus aureus*, *Propionibacterium acnes* and *Rhodococcus equi* and against gram negative bacteria represented by *Pseudomonas mosselii* dan *Escherichia coli.* There were 37 out of 64 extracts that showed antibacterial activity as indicated by clear (inhibition) zone around the disk dropped with extract (Tabel 3). Only extracts that produced inhibition zone mentioned in the Table. Based upon inhibition index value obtained, root of *R. apiculata* (sample code 74) showed inhibition zone on agar media with gram positive bacteria *P. acnes* for all four solvents (74H, 74Ea, 74Et dan 74A) and extracts 74Ea and 74Et on media with *R. equi*. Sample 76Et also produced inhibition zone on agar media with *P. acnes* and *R. equi* bacterial strains.

Tabel 3. Bacterial inhibition zone (mm) and inhibiton index of extracts.

No.	Sample code	Bacterial strains	Disk diameter + inhibiton zone (mm)		Inhibition index	
			Positive control	Samples	Positive control	Samples
1	72H	*P acne*	15.95	7.54	1.6583	0.2567
		E coli	25.88	7.26	3.133	0.2100
2	72A	*S aureus*	17.32	7.11	1.8875	0.1850
3	72Et	*E coli*	25.88	6.94	3.3133	0.1567
4	73H	*R equi*	20.4	6.21	2.4	0.0350
5	73Ea	*R equi*	25.36	6.77	3.2275	0.1275
6	73Et	*P acne*	25.66	6.50	3.2767	0.0833
7	73A	*P acne*	25.66	6.99	3.2767	0.1650
8	74H	*P acne*	15.95	6.17	1.6583	0.0283
9	74Ea	*P acne*	11.25	6.29	0.8750	0.0483
		R equi	25.36	7.98	3.2275	0.3300
10	74Et	*P acne*	28.74	6.87	3.7892	0.1450
		R equi	23.48	7.91	2.9125	0.3183
11	74A	*P acne*	28.74	7.49	3.7892	0.2483
12	75Ea	*R equi*	25.36	6.44	3.2275	0.0733
13	75A	*S aureus*	16.90	6.43	1.8175	0.0717
14	76H	*P acne*	12.49	6.90	1.0817	0.1500
15	76Ea	*P acne*	17.88	6.89	1.9800	0.1483
		P mosselii	11.63	7.59	0.9383	0.2650
16	76Et	*P acne*	23.32	7.30	2.8858	0.2167
		R equi	25.41	6.52	3.2342	0.0867
	77Ea	*S aureus*	31.6	7.74	4.2667	0.2900
17		*E coli*	34.40	17.39	4.7325	1.8983
		P mosselii	11.63	7.89	0.9383	0.3150
18	77Et	*P acne*	31.6	7.02	4.2667	0.1700
19	77A	*P acne*	31.6	7.96	4.2667	0.3267
		E coli	34.40	7.71	4.7325	0.2850
20	79Ea	*R equi*	20.81	6.48	2.4683	0.0800
21	79A	*S aureus*	17.32	8.47	1.8875	0.4117
22	80H	*S aureus*	30.31	6.79	4.0517	0.1317
23	80Ea	*R equi*	20.81	7.25	2.4683	0.2083
24	81H	*R equi*	21.80	6.68	2.6325	0.1133
25	81Ea	*P mosselii*	11.88	6.25	0.9792	0.0417
26	82H	*P acne*	11.85	6.91	0.9750	0.1517
		R equi	21.80	7.13	2.6325	0.1883
	83H	*E coli*	36.31	7.66	5.0517	0.2767
27		*P mosselii*	10.46	7.60	0.7442	0.2667
		R equi	21.80	6.92	2.6325	0.1533
28	83Ea	*S aureus*	27.50	6.59	3.5825	0.0983
		E coli	36.31	6.85	5.0517	0.1417
29	83Et	*R equi*	23.40	7.80	2.8992	0.3000
30	84H	*P acne*	11.85	6.87	0.9750	0.1450
		R equi	21.80	6.80	2.6325	0.1333
31	84A	*S aureus*	16.90	10.72	1.8175	0.7867
32	85H	*P acne*	21.04	6.41	2.5067	0.0683
		R equi	19.37	7.78	2.2283	0.2967
33	86H	*R equi*	19.37	6.81	2.2283	0.1350
34	87H	*R equi*	19.37	6.49	2.2283	0.0817
35	88H	*R equi*	19.37	6.66	2.2283	0.1100
36	88Et	*P acne*	24.78	8.39	3.1292	0.3983
37	88A	*P acne*	24.78	7.58	3.1292	0.2633

Figure 1 showed an inhibition zone formed due to the addition of extract of root of *B. gymnorrhiza* (77Ea) on agar media with gram negative bacteria *E. coli*. The formed inhibition zone was the largest one with diameter 17.39 mm with inhibition index value of 1.8983.

Extracts 77Ea also produced inhibition zone as large as 7.89 mm in diameter on agar media with *P. mosselii* and *S. aureus*. Extracts 76H, Ea and Et showed inhibition zone on agar media with *P. acnes, P. mosselii* and *R. equi*. The second highest inhibition index was 0.7867 recorded for leaf of water extract of *Avicennia marina* (84 A) screened against *Staphylococcus aureus.* Taken altogether, these data strongly suggested that mangrove extracts used in this study were potential as antibacterial agents with inhibition index value from the lowest to the highest were 0.0283 and 1.8983, respectively.

Previous studies reported that mangrove extracts had shown their activity against microbes or pathogen parasites in animals and plants [8,9] including HIV [10] and Hepatitis-B virus [11]. Phytochemical qualitative analysis showed that most if not all extracts contain saponin and tannin in considerable amount (Tabel 4). The two phytochemical constituents and flavonoid had shown their activity against some bacteria [12]. Samples collected contained flavonoid and steroid in less amount and no alkaloid detected in almost all samples.

It is important to note that chemical constituents and bioactivity of mangrove extracts and plants in general vary depend upon not only from species to species but also due to geographical conditions. This is also important to identify factors contributing to bioactivity, such as season, location and reproduction cycle stage [13]. Therefore, documentation of samples collection includes taxonomy, time and location, collector either individual or institution and species availability. This will be very helpful in tracing and sample monitoring during research process for accessibility purpose and benefit sharing and recollection.

Considering the very large area covered by mangroves in Indonesia and worldwide, mangrove research, particularly for the purpose of drug discovery is still very limited. This opens up opportunities for researches to start putting their efforts individually and collaboratively on mangrove research which also applies to mangrove's associates.

Tabel 4. Phytochemical qualtitative analysis of mangrove extracts.

Sample code	Alkaloid			Triterpenoid	Steroid	Quinon	Flavonoid	Saponin	Tanin
	Mayer	Wagner	Dragendof						
72	-	-	-	-	++	-	-	+	++
73	-	-	-	+	-	-	-	+++	++
74	-	-	-	-	-	-	+	+++	+++
75	-	-	-	-	++	-	+++	+++	+++
76	-	-	-	+	-	+	+	+++	++
77	-	-	-	-	-	-	-	+++	+++

79	-	-	-	-	++	-	+	+++	+++
80	-	-	-	-	-	+	-	+++	+++
81	-	-	-	-	-	+	+++	+++	++
82	-	-	-	-	+++	-	-	+++	+
83	-	-	-	-	+	-	-	-	+++
84	-	-	-	-	+	-	++	++	++
85	-	-	-	-	+	-	-	+	-
86	-	-	-	-	+	-	-	+++	+
87	+	+	+	-	+	-	+	+	++
88	-	-	-	-	+	-	++	-	+++

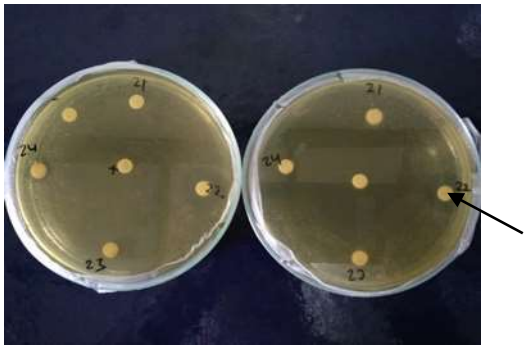

Figure 1: The largest inhibition zone formed due to the addition of extract of root of *B. gymnorrhiza* (77Ea) on agar media with gram negative bacteria *E. coli* with diameter 17.39 mm and inhibition index value of 1.8983 (as indicated by arrow).

Experimental

Plant materials: Mangroves plants were collected from the Eastern Coastline of Lampung Province, Indonesia in May 2017. There were eight species of mangroves used in this study with the total of 16 samples collected from different parts of plants such as leaves, barks and roots (Tabel 5). The eight species were identified for confirmation at the Herbarium of the Center for Biological Research of the Indonesian Institute of Sciences, Bogor, Indonesia.

Table 5: Mangroves species and part of the plants used in this study*.

Name of Plants	Part of Plants	Samples Code
Rhizophora apiculata	Leaf	72
	Bark	73
	Root	74
Bruguiera gymnorrhiza	Leaf	75
	Bark	76
	Root	77
Rhizophora mucronata	Leaf	79
	Bark	80
	Root	81
Thespesia populnea	Leaf	82
	Fruit	83
Avicennia marina	Leaf	84
	Root	85
Xylocarpus granatum	Leaf	86
Ceriops tagal	Leaf	87
Sonneratia alba	Leaf	88

*Part of plants collected were based upon the nature of the plants.

Solvents and chemicals: Distilled water, ethanol, *n*-hexane, ethyl acetate were obtained from Brata Chem. Concentrated HCl, *n*-amyl alcohol, acetone, dichloromethane, Liebermann-Burchard reagent, chloroform-ammonia, H_2SO_4 2 M, Mayer, Dragendorf and Wagner reagents, Magnesium powder, $FeCl_3$ 1%, NaOH 10% and DMSO 20% were obtained from Sigma-Aldrich.

Bacterial strains, media and antibiotic: *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Pseudomonas mosselii* and *Rhodococcus equi* bacterial strains were all purchased from the Indonesia Culture Collection (InaCC), nutrient agar (NA), Tryptic Soy Broth (TSB), Whatmann filter (6.02 ± 0.02mm) and tetracycline.

Simplisia water content [14]**:** Empty porcelain dish was dried in the oven at 105 °C in 30 minutes and cooled in desiccator in 30 minutes and weighed. One gram of sample was weighed into the dish, heated in the oven at 105 °C for certain period of time. The steps were redone until constant weight was obtained. Water content was determined by using the following equation:

Water content = $\frac{B-A}{B} \times 100\%$; A = dry sample weight (g), B = sample weight (g)

Maceration extraction: Extraction method used was gradient maceration [7]. Four different solvents were used, which were *n*-hexane, ethyl acetate, ethanol and water. Ratio between solvent and simplisia was 5:1 with overnight maceration, 3:1 with maceration for 17 and 7 hours. Extracts separated from their residues was concentrated with rotary evaporator. The yields were then determined based on the ratio of concentrated extract weight with initial sample weight. Extractions were performed triplicate.

Antibacterial activity: Disk diffusion method was used to determine antibacterial activity of mangrove extracts [15]. Media used for *Escherichia coli* strain was Nutrient Agar (NA). Media used for *Staphylococcus aureus*, *Propionibacterium acnes*, *Pseudomonas mosselii* and *Rhodococcus equi* strains was Tryptone Soya Agar. All bacterial strains were subcultured in Triptic Soy Broth and incubated at 37 °C overnight. Sterilized agar media was prepared. Bacterial suspension was mixed with the agar and solidified for 5 minutes. Disk paper with the size of 6 mm in diameter was placed on the solid agar media with tweezers and was dropped with 20 µL of 1% extract in 20% DMSO solvent. Tetracycline was used as positive control. Bacterial cultures were incubated at 37 °C overnight. Inhibition zone diameter formed was measured in millimeter (mm). Bacterial inhibition index value was calculated by using the following equation:

$$\text{Inhibiton index} = \frac{\text{d inhibition zone} - \text{d disk}}{\text{d disk}}; \text{ d = diameter}$$

Qualitative phytochemical analysis [16]: Alkaloids test was performed by mixing 4 ml of chloroform-ammonia mixture with 0.1 gram crude extract and was then filtered. Few drops of H_2SO_4 2 M was added into the filtrate and mixed until two layers formed. Transparent layer (acidic layer) was divided into 3 reaction tubes. Mayer, Wagner and Dragendorf reagents were added into each tubes. Positive alkaloids test results indicated by the formation of white, brown or red precipitation by addition of Mayer, Wagner or Dragendorf reagents, respectively.

Triterpenoid and steroid tests were performed by heating mixture of 0.1 gram crude extract with 5 ml ethanol at 50 °C and then filtered. The filtrate was then concentrated and dissolved with ether. The ether layer was dropped on a drop plate and air-dried. Few drops of Liebermann-Burchard reagent (concentrated H_2SO_4 and CH_3COOH anhydrate) was added onto the drop plate. Positive triterpenoid test result indicated by the formation of red color and positive steroid test results indicated by the formation of green or blue color.

Phenolic and flavonoid tests were performed by mixing 0.1 gram of crude extract with 5 ml of distilled water and then boiled for 2 minutes and filtered. NaoH 10% was added into 2 ml of filtrate for

phenolic test. Red color indicates that phenolic compounds are present in the sample. The presence of flavonoid compounds can be detected by mixing 0.1 gram Magnesium powder, 1 ml of concentrated HCl and 1 ml of amyl alcohol with 2 ml of the filtrated. The formation of red, yellow or orange color indicate a positive result.

Saponin and tannin tests were performed by mixing 0.1 gram of crude extract with 5 ml distilled water and then boiled and filtered. Filtrate was divided into 2 reaction tubes. Saponin test was done by cooling the filtrated and mixed until foam formed. Positive result indicated by the formation of foam that lasts for about 10 minutes. Tannin test was done by mixing the filtrate with $FeCl_3$ 10% solution. Positive result indicated by the formation of dark blue or blackish green color.

Typically this section should be divided into subsections, the contents of which varies according to the subject matter of the article. This must contain all the information to guarantee reproducibility. In an introductory paragraph, special equipment, etc. should be detailed. A precise workup containing all details, e.g., the amount of solvent used for extraction, details of chromatographic purifications and yields etc., should be given. Physical and spectroscopic data can be included in the experimental section or in tabular form.
Spectroscopic data should be stated in the order and format shown in following examples:

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Original Research Article

Methods Development for Mangrove Extraction towards Standardization of Indonesian Extract Library

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abstract

Natural resources, plants in particular, have been utilized by human for different purposes including as medicines. Drug discovery processes can be accelerated through a large collection of extracts in numbers, quality and quantity. By virtue of its vast numbers of biodiversity, developing a large collection of extracts (extract library) for drug discovery in Indonesia is very plausible thing to do. Due to the variations of chemical constituents of a plant, a reproducible method of extraction and profiling should be developed. The aim of this study is to develop some methods for extraction and profiling of mangrove plants. The methods will be used as the basis for development for Indonesian extract library especially for drug discovery and cosmetics. In this study, mangrove plants had been chosen due to their availability and potential as medicines. Several factors that could possibly determine chemical constituents were taken into consideration. These include but not limited to location, date and time, packaging, storage, species identification, solvents, extraction method and fingerprint profiling. Stability testing should also be conducted for mid and long-term storage. Extraction methods used in this study were maceration and ultrasonic assisted extraction by employing various solvents representing different polarity such as water, alcohol, ethyl acetate and hexane. Thin layer chromatography method was used for fingerprint profiling. In the future, implementation of barcoding system and integration of all data into a comprehensive information system is necessary. This can be utilized as a window of the Indonesian extract library as a whole.

1. Introduction

Nature has been known for centuries as invaluable sources for medicines. Traditional medicines originated from plants in particular have been practiced in countries such as China, India and Indonesia (Mittermeier et al., 2005). Indonesia for example possesses approximately 30,000 vascular plants which comprises about 10 percent of world's plants (Royal Botanical Garden Report, 2017). According to a report from 1980 until 2014, about 50 percent of drugs recognized by the Food and Drug Administration (FDA) of the United States of America and other similar organizations were originated from natural products or their derivatives (Newman and Cragg, 2016). In fact, for certain disease such as cancer, 75% of the drugs were originated from natural products (Newman and Cragg, 2016). By virtue of the richness in biodiversity, drug discovery process in Indonesia can be accelerated by having a large collection of extracts of natural products in large numbers (designated as extract library). This work was aimed to develop some methods to establish a protocol for preparation of extract library that should meet some criteria.

Adequate information of extracts should be obtained, so that extracts can be collected and stored in extract library repository for further usage such as drug screening purpose. Several important aspects that might contribute to the quality and quantity of chemical constituents of extracts were taken into consideration. These aspects include but not limited to geographical location, date and time, sample packaging, sample storage, method of extraction, solvents and fingerprint profiling. The effect of geographical location to chemical constituents of plants had been reported in different studies (Khattak, KF and Rahman, TR, 2015; Liu, W. *et. al.*, 2016; Margraf, T. *et. al.*, 2016). Stability testing should also be conducted for mid and long term storage. Additional information such as bioactivity of the extracts against certain disease(s) will be very useful. While crude extracts can be stored in any laboratories, all information accompanying the extracts eventually should be stored in a well-developed and maintained databases and can be easily accessed for future references.

In this study, mangrove plants were utilized as samples for methods development to establish the protocols for Indonesian extract library. This is because mangroves and their associates are very potential medicinal plants (Bandaranayake, 2002). Indonesia is home of about 20 family of mangroves and hundred species of their associates which comprises of approximately 23% of total world mangrove forests (Giri *et al.*, 2011).

2. Materials and Methods

In order to standardize the protocols for extract library repository, particular information need to be obtained from a biological material. Particular information such as geographical location, date and time, extraction, fingerprint profiling, and species identification are some of important aspects to be taken into consideration. Methods of choice will also vary depend upon biological materials to be extracted and or analyzed. For example, material handling for plant originated from marine will be different from land (Mccloud, 2010). This include but not limited to harvesting, packaging or storage.

2.1 Solvents and chemicals

Distilled water (dH_2O), ethanol (EtOH), *n*-hexane (Hex), and ethyl acetate (EtOAc) were obtained from Brata Chem. Concentrated HCl, *n*-amyl alcohol, acetone, dichloromethane, Liebermann-Burchard reagent, chloroform-ammonia, H_2SO_4 2 M, Mayer, Dragendorf and Wagner reagents, Magnesium powder, $FeCl_3$ 1%, NaOH 10% and DMSO 20% were obtained from Sigma-Aldrich.

2.2 Plant materials and experimental conditions

There were eight species of mangroves used in this study which were obtained from the Pasir Sakti Village in the Eastern Coastline of Lampung Province, Indonesia. The samples were collected from different parts of the plants such as leaves, barks or roots. The samples were designated as listed in Table 1.

Table 1: Mangroves species and part of the plants used in this study*.

Name of Plants	Part of Plants	Samples Code
Rhizophora apiculate	Leaf	72 (1)
	Bark	73 (2)
	Root	74 (3)
Bruguiera gymnorrhiza	Leaf	75 (4)
	Bark	76 (5)
	Root	77 (6)
Rhizophora mucronata	Leaf	79 (7)
	Bark	80 (8)
	Root	81 (9)
Thespesia populnea	Leaf	82 (10)
	Fruit	83 (11)
Avicennia marina	Leaf	84 (12)
	Root	85 (13)
Xylocarpus granatum	Leaf	86 (14)
Ceriops tagal	Leaf	87 (15)
Sonneratia alba	Leaf	88 (16)

*Part of plants collected were based upon the nature and the availability of the plants.

2.3 Sample packaging, storage and transport

After harvesting at the collection site, the samples were first wrapped with paper and placed in plastic bags (Harcourt, 2015) and put in a cardboard box. This will protect the samples from heat to prevent moist loss during transport. The samples were then transported to the Tropical Biopharmaca Research Center LPPM IPB, Bogor. Upon arrival, the samples were stored in a freezer at -20°C.

2.4 Species identification

The eight species were identified for confirmation at the Herbarium of the Center for Biological Research of the Indonesian Institute of Sciences, Bogor, Indonesia. A combination of at least two parts of a plant should be provided for minimum requirement for plant identification. The combinations could consist of bark, flower, fruit, leaf, root, seed or stem.

2.5 Sample preparation

Preparation of sample was carried out using an oven with a temperature of less than 50 °C until the samples were dry. The time needed for leaves was approximately 3 days, while the roots and stems were about 6 days to dry. The dried samples were stored in plastic and the moisture was kept and observed. Unless otherwise stated, the protocols for preparation in this study refer to the Indonesian Herbal Pharmacopeia (Department of Health of the Republic of Indonesia., 2009).

2.6 Water content determination

The procedure for determining the water content of dried samples was carried out using a moisture balance. Five grams stored in an oven at 105°C for 5 hours, then weighed. The difference of mass before and after heating is the value of the water content of sample (Sulasmi et al., 2016).

2.7 Qualitative Phytochemical analysis

Phytochemical tests in this study include qualitative tests of alkaloid, phenolic, triterpenoid/steroid, and hydroquinone (Harborne, 1987).

2.8 Extraction

Extraction method used was maceration according to the Indonesian Herbal Pharmacopeia (Department of Health of the Republic of Indonesia., 2009). Four different solvents were used, which were *n*-hexane, ethyl acetate, ethanol and water. Ratio between solvent and dried sample was 5:1 with overnight maceration, 3:1 with maceration for 17 and 7 hours. Extracts separated from their residues and concentrated with rotary evaporator. The yields were then determined based on the ratio of concentrated extract weight with initial sample weight. Extractions were performed triplicate.

2.9 Thin Layer Chromatography-Bioautography

Thin layer chromatography (TLC)-bioautography was performed to obtain additional information regarding bioactivity of the fractions of an extract. TLC-bioautography was used to determine active bands as antibacterial before fractionation using column chromatography. The results of the TLC-bioautography can be used as a guide to recognize active fractions that have antibacterial activity. This method is relatively fast, easy to perform, and economical in using extracts. TLC-bioautography was carried out by attaching the TLC plates which had been eluted over the bacterial suspension medium. TLC was left for 24 hours to diffuse chemical components into the media (Yulianty *et. al.*, 2011). The activity of bacterial growth inhibition was characterized by the formation of specific clear zones on certain bands. The Rf values in the band was used as a reference for the isolation of antibacterial active compounds.

3. Results

3.1 Sampling and Preparation

The extraction results not only depend upon the types of biota such as species of plants or any other sources of natural products, but also depend upon other factors such as location and time. These conditions will determine on how plants or other natural sources generate secondary metabolites. The production of secondary metabolites is strongly influenced by seasons, environmental conditions, age and type of organ (Sampaio *et al.*, 2016). Thus, it is very important to record all necessary information related to those factors mentioned above. This is also to ensure that uniformity of samples for future need is well maintained. Species identification in particular, is one of key factors that needs to be performed. The time between sampling, temporary samples storing, samples storage in the freezer and simplisia preparation must be kept to minimum. The duration from sampling to simplisia preparation will influence the results of the extracts which related to the intercellular enzyme activity of the sample itself and the activity of microbes (bacteria and fungi attached to the sample). The recording of sampling details until the storage of simplisia needs to be recorded properly so that it can be used as a reference if re-collection is needed.

The use of extraction methods was determined by several things, namely cost efficiency, time, and extraction results. It is necessary to optimize the extraction so that the extraction yield can reach optimum results in terms of quantity and quality and to achieve an adequate amount of extract. It is also necessary to develop a method for storage of the extract which is related to the effectiveness of screening. An ideal storage must include a long shelf life and consistent extract quality so it can produce more representative results from many screening processes for various types of diseases. All samples that have been successfully collected are summarized in Table 2. All samples are coded and dried. The number of samples obtained varies according to the availability of samples at the sampling location. The largest shrinkage of drying is found in the leaves of all parts of the plant, while the smallest drying shrinkage is found in the stem.

Table 2: sample supply used in this study

Samples	Part	Shrinkage (%)	Used Amount (g)	Water Content (%)
R. apiculate	Leaf	78	50.01 50.03	8.60

			50.01	
			50.10	
	Stem	47	50.02	2.30
			50.06	
			50.01	
	Root	62	50.01	7.36
			50.05	
			50.02	
	Leaf	63	50.03	
			50.02	5.34
			50.00	
B. gymnorrhiza	Stem	51	50.00	3.57
			50.01	
			50.01	
	Root	60	50.01	8.23
			50.00	
			50.01	
	Leaf	71	50.01	4.78
R. mucronata			50.01	
	Stem	47	-	4.85
	Root	60	-	7.56
			50.01	
T. populnea	Leaf	71	50.01	7.54
			50.00	
			50.01	
	Leaf	77	50.01	9.43
A. marina			50.01	
			50.02	
	Root	71	50.04	-
			50.02	
			50.01	
X. granatum	Leaf	80	50.01	8.98
			50.01	
			50.01	
C. tagal	Leaf	82	50.01	9.45
			50.05	
			50.00	
S. caseolaris	Leaf	84	50.01	7.23
			50.01	

3.2 Water content determination

Water content of sample (Table 2) were kept below 10% which is a suitable percentage for simplisia analysis according to the Indonesian Herbal Pharmacopeia (Department of Health of the Republic of Indonesia., 2009).

3.3 Qualitative Phytochemical analysis

Each simplisia does not always produce a positive reaction to all tests. This shows that mangrove plants are quite rich in secondary metabolites and the composition is not the same one to another (Joel and Bhimba, 2013). Furthermore, the results of this test can be a simple assumption for continued antibacterial and antitumor screening. The following results of the dried sample phytochemical test were presented in Table 3.

Table 3. Phytochemical qualitative analysis of samples

Sample code	Alkaloid		Dragendof	Triterpenoid	Steroid	Quinon	Flavonoid	Saponin	Tanin
	Mayer	Wagner							
72	-	-	-	-	++	-	-	+	++
73	-	-	-	+	-	-	-	+++	++
74	-	-	-	-	-	-	+	+++	+++
75	-	-	-	-	++	-	+++	+++	+++
76	-	-	-	+	-	+	+	+++	++
77	-	-	-	-	-	-	-	+++	+++
79	-	-	-	-	++	-	+	+++	+++
80	-	-	-	-	-	+	-	+++	+++
81	-	-	-	-	-	+	+++	+++	++
82	-	-	-	-	+++	-	-	+++	+
83	-	-	-	-	+	-	-	-	+++
84	-	-	-	-	+	-	++	++	++
85	-	-	-	-	+	-	-	+	-
86	-	-	-	-	+	-	-	+++	+
87	+	+	+	-	+	-	+	+	++
88	-	-	-	-	+	-	++	-	+++

3.4 Extraction

The yield of extracts of the 16 samples were between 0.14% (highlighted green) to 26.23% (highlighted yellow) that belonged to *n*-hexane extract of root of *Rhizophora mucronata* and water extract of leaf of *Avicennia marina*, respectively as shown in Table 4. The data clearly showed that root extract using non-polar solvent (*n*-hexane) resulted in lower yield percentage compared to more polar solvents (samples 74, 77, 81 and 85). This indicated that root sample contains less nonpolar constituents compared to other parts of plants (Harcourt, 2015). Different method such as ultrasound-assisted extraction can also be used for extracting mangrove samples (Audah *et.al*, 2018). However, due to the limitation of the equipment, maceration extraction was still favorable as the method of choice in this study.

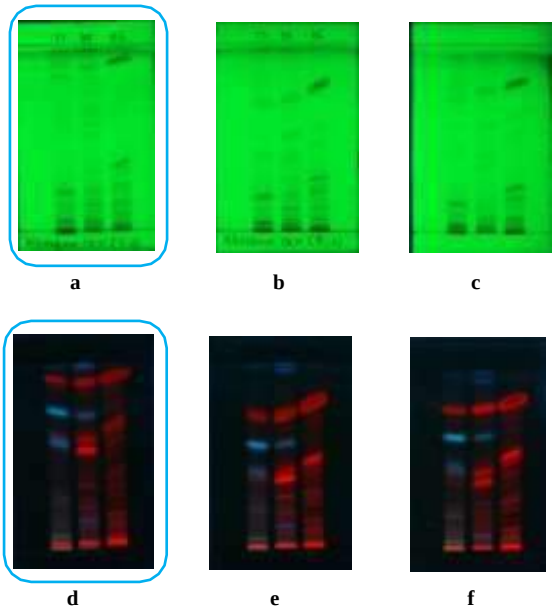
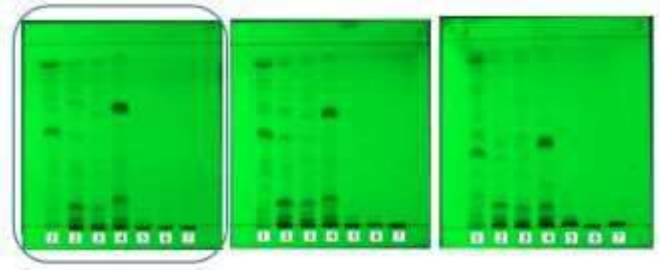


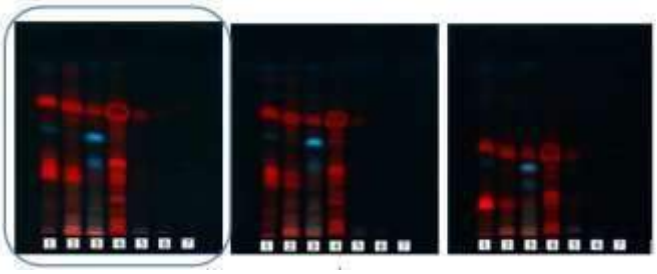
Figure 1. Chromatogram of ethyl acetate fraction of Xylocarpus granatum, Xylocarpus granatum leaves, and Avicennia marina. Mobile phase = Chloroform: Dichloromethane (v / v) by comparison (a,d) 9: 1, (b,e) 8: 2, and (c,f) 7: 3. UV 254 nm (top) and UV 366 nm (bottom).

Table 4. Yield of extracts (%)*

Sample code	Yield	Sample code	Yield	Sample code	Yield	Sample code	Yield
72H	1.28	76H	0.26	81H	0.14	85H	0.5
72Ea	3.22	76Ea	0.27	81Ea	0.15	85Ea	0.89
72Et	6.54	76Et	2.3	81Et	3.6	85Et	1.98
72W	16.2	76W	5.45	81W	2.53	85W	10.31
73H	0.42	77H	0.29	82H	2.46	86H	0.76
73Ea	0.43	77Ea	0.32	82Ea	2.73	86Ea	1.79
73Et	3.3	77Et	8.95	82Et	3.55	86Et	6.94
73W	1.84	77W	4.14	82W	15.1	86W	21.18
74H	0.25	79H	1.04	83H	3.08	87H	2.41
74Ea	0.25	79Ea	2.36	83Ea	3.64	87Ea	2.27
74Et	11.92	79Et	2.48	83Et	2.99	87Et	10.86
74W	6.31	79W	13.26	83W	13.4	87W	19.58
75H	2.6	80H	0.26	84H	1.7	88H	1.52
75Ea	1.34	80Ea	0.23	84Ea	1.4	88Ea	1.43
75Et	7.09	80Et	4.88	84Et	7.42	88Et	9.46
75W	20.3	80W	3.72	84W	26.2	88W	7.06

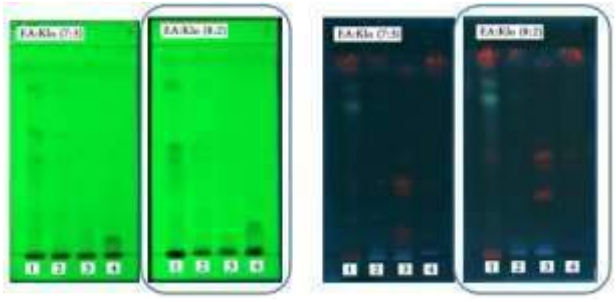
*Solvents abbreviation: H = n-hexane, Ea = ethyl acetate, Et = ethanol, W = water.





d e f

Figure 2. Chromatogram of (1) 74 H, (2) 74 EA, (3) 77 EA, (4) 86 EA, (5) 74 ET, (6) 74 W, (7) 88 W. Mobile phase: Chloroform: Dichloromethane (v/v) by comparison (a,d) 9:1, (b,e) 8:2, and (c,f) 7:3. UV 254 nm (top) and UV 366 nm (bottom).



a b

Figure 3. Chromatogram of (1) 74Et, (2) 74A, (3) 88A, (4) 84W. Mobile phase: Ethyl acetate: chloroform (v/v) with 7:3 and 8:2 comparisons. (a) UV 254 nm, (b) UV 366 nm.

3.5 Thin Layer Chromatography-Bioautography

It is important to note that the elution results may vary from species to species as well as location and time. Therefore, whatever the results will be, recording all the information become critical for validity of the extract library stored in data repository. The presence of inhibitory activity against bacteria was characterized by the appearance of zones or specific clear areas on the band (R_f). The bioautogram showed that there was at least one inhibition zone detected. The inhibition zone was found at the R_f 0.18 (Figure 4). The R_f value in the band is used as a reference for the isolation of antibacterial active compounds (Patra *et al.*, 2012). The isolation process is going to be carried out by fractionation using column chromatography.

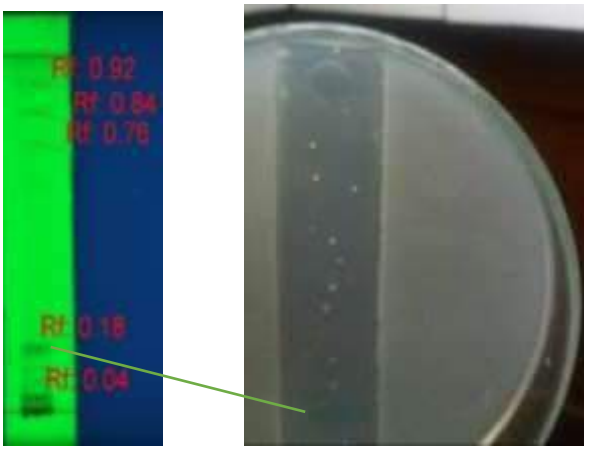


Figure 4. TLC-Bioautogram contacts Xylocarpus granatum leaves ethyl acetate fraction against *E. Coli* and with information on the R_f value under UV light 254 nm.

4. Discussion

The advancement of sciences and technology does not always positively correlate with the ability of human to overcome healthcare issues such as the availability of medicines for particular diseases. As a matter of fact, new diseases either communicable or non-communicable ones, emerge even before drugs for the existing diseases discovered. The World Health Organization once reported that approximately 30 new diseases emerge in every 20 years (World Health Report, 1996). Healthcare issues become more challenging by the increasing number of antibiotics resistant and reemerging of microbial pathogens. Recently, the WHO issued a list of 12 bacteria for which new antibiotics are urgently needed as shown in table 5 (WHO, 2017). Antimicrobial resistance is also on the rise in Indonesia. For example, epidemiological data in 2001 on *E. Coli* from rectal samples showed remarkably high resistance to several antibiotics (Parathon, 2017). In 2010, there was some evidences from several patients in Semarang, Indonesia that showed a resistance of antibiotics against *S. pneumonia* (Farida et al., 2014). Misuse and overuse of antibiotics in humans, livestock and aquaculture are believed to be the main causes of resistance in the country. Taken altogether, these could give a tremendous burden and threat not only to an estimated population of more than 260 million of Indonesian people, but also to the world population as a whole.

Table 5. WHO priority pathogens list for R&D of new antibiotics

Type of Priority	Pathogens	Description
Critical	*A. baumannii*	carbapenem-resistant
	P. aeruginosa	carbapenem-resistant
	Enterobacteriaceae	carbapenem-resistant, ESBL-producing
High	*E. faecium*	vancomycin-resistant
	S. aureus	methicillin-resistant, vancomycin-intermediate and resistant
	H. pylori	clarithromycin-resistant
	Campylobacter spp.	fluoroquinolone-resistant
	Salmonellae	fluoroquinolone-resistant
	N. gonorrhoeae	cephalosporin-resistant, fluoroquinolone-resistant
Medium	*S. pneumoniae*	penicillin-non-susceptible
	H. influenzae	ampicillin-resistant
	Shigella spp.	fluoroquinolone-resistant

To overcome these challenges, researchers should come up with a solution on how to find drugs in most effective and efficient ways in terms of time and cost. Because conventional drug discovery is a lengthy and expensive processes. It can cost as much as USD 1 billion and can take as long as 12-15 years period of time to discover for one drug only (Hughes, J.P. *et. al.*, 2011).

Nature has been known almost as long as human civilization as a priceless sources for all human needs including medicine. Therefore, the exploration of biodiversity could be an answer to current healthcare issue in providing medicines for different types of diseases. This approach is very suitable with Indonesian condition which is well known as one of the richest country in the world in terms of biodiversity (Royal Botanical Garden, 2017). One of the developments to discover novel therapeutic drugs is through screening process of natural products. This work is to propose a legitimate approach based upon the Indonesian biodiversity which is to develop what so called the Indonesian Extract Library. The main purpose of the Indonesian Extract Library is to synergize and integrate all efforts in searching of medicines from Indonesian biodiversity. Eventually, all information related to collected extracts will be integrated into a comprehensive information system. Similar

works had been done in some countries like Australia through the Department of Defense (DSTO, 2008) and the United States of America through the National Institute of Health under the National Center for Complementary and Integrative Health (NCCIH).

In this study, several methods have been tested to formulize a definitive protocols for preparation of extract library. This can be used as initial steps towards standardization of the Indonesian Extract Library. By establishing a protocol for preparation of extract library, it is hoped that drugs discovery in the future can be more effective and efficient. In addition, it can also reduce the production cost during drugs discovery and development. Mangroves had been chosen as plant materials of interest due to their potential as medicines. Mangroves and mangrove's associates are very potential medicinal plants (Bandaranayake, 2002). Besides, mangroves are also very easily found along approximately 90,000 kilometers of Indonesian coastline. The availability of the materials is one of important aspects for continuity and sustainability of drugs research and development. Although for commercial purpose, synthetic biology will be more plausible in ensuring continuity of the materials for the long run. The eight mangroves species selected in this study were based on traditional uses by local people (local wisdom) and their availability in the area.
During sampling, several aspects such as seasons, environmental conditions, age, and type of organ should be taken into consideration. This step is crucial due to the fact that extract library is closely related to how plants or other natural sources generate secondary metabolites. The secondary metabolites and also therapeutic efficacy by medicinal plants can be influenced by those factors (Sampaio *et al.*, 2016; Ahmad *et al.*, 2011; Szakiel and Henry, 2011).

Samples packaging either during transportation or storage prior to extraction should be handled properly. This is to minimize the moisture loss and or oxidation of the sample during the shipping and or storage. Moisture loss and or oxidation can lead to damage or change in chemical composition of the samples especially volatile compounds or compounds function as antioxidants or microbial quality of samples (Ajayi *et. al.*, 2015; Bakan and Eksi, 2014). Mangroves are plants which are rich in antioxidants as shown in table 3. These findings were similar to previous studies that mangroves had high antioxidant contents (Audah *et. al.*, 2018; Rohaeti *et al.*, 2010). Some enzyme activities might affect the chemicals or metabolites composition of samples during storage (Sharma *et al.,* 2016). Therefore, it is important that the samples should be stored in a freezer at -20°C upon arrival.

In preparation of simplisia, water content also can determine the quality of simplisia (Table 2). The water content should be less than 10% with the characteristics of simplisia is easy to break, not mouldy and smells like fresh materials in order to have high quality simplisia (Setiafianti, *et. al.*, 2017). Methods of extraction including solvent of choices will vary depend upon several factors that include but not limited to type of samples, compounds to be obtain and availability of the instruments. The most important thing of all is to record whatever methods used. Because eventually, the determining factor will be the elution steps which is required to obtain the best separation of compounds to be fractinated. Bioactivity of extracts or fractions is very useful information to have in the extract library repository. This information will help other researchers to select certain extracts or fractions for further studies such as for drug screening. Large collection of extract allows high throughput screening method to be applied. The concept of extract library was firstly introduced when pharmaceutical companies firstly implemented high throughput screening concept (Pereira dan Williams, 2007).

Mangroves as many of other plants contain various chemical compounds as shown in table 3. The success for fractionation will depend on the suitable solvents with the best combination of mixture. However, the application of the fractions themselves will depend on the right match between the compounds against particular diseases. In other words, all fractions or compounds either polar or non-polar should be discarded. The results of this work had shown that some extracts or fractions were effective as antibacterial as shown in Figure 4 and our previous works (Audah *et. al.*, 2018). While some others were active as anticancer (unpublished results).

In conclusions, the methods and the results presented in this work suggested that developing extract library is a very plausible and important thing to do. A large collection of extracts or fractions or even single compounds can be easily obtain by exploring potential medicinal plants such as mangrove, mangrove associate and other forms or natural biological sources. This report can be used as stepping stone towards standardization for the development of Indonesian extract library from Indonesian biodiversity. Eventually, implementation of barcoding system and integration of all data and information into a comprehensive information system is necessary and can be utilized as a window of the Indonesian extract library as a whole.

Conflicts of interest

The authors declare that there is no conflict of interest on this research.

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Development of extract library from indonesian biodiversity: exploration of antibacterial activity of mangrove *bruguiera cylindrica* leaf extracts

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Abstract. Antibacterial drugs derived from natural sources play significant roles in the prevention and treatment of bacterial infections since antibiotics have become less effective against many infectious diseases. Mangroves are very potential natural antibacterial sources among great numbers of wild medicinal plants. *Bruguiera cylindrica* is one of the many mangroves species which spread along Indonesian coastline. The aim of this study was to explore the antibacterial activity of *B. cylindrica* wet and dried leaf extracts. The wet extracts study was conducted with three different solvents system (water, ethanol, and n-Hexane) against *Escherichia coli* and *Staphylococcus aureus*. While, the dried extracts study was conducted with four different solvents system (water, ethanol, chloroform and n-Hexane) against three types of bacteria, *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The study showed that ethanol was the best solvent for extraction of phenolic and flavonoid. Antibacterial actitivity was measured by zone of inhibition which obtained from agar-disk diffusion method. The widest area of zone of inhibition was showed by wet extracts with ethanol against *S. aureus* and E. coli are 14.30 and 13.30 mm, respectively. While, the zone of inhibition dried extracts with ethanol against *S. aureus*, *S. epidermidis* and *E. coli* are 9.32, 6.59 and 6.20 mm, respectively. In conclusion, both type of extracts showed significant antibacterial activity against gram-positive bacteria as crude extracts.

Keywords. antibiotics, antimicrobial, infectious diseases, medicinal plants, natural products

1. Introduction

Antibiotics are the most important drugs for fighting bacterial infections [1,2]. However, antibiotics have become less effective against many infectious diseases not only because many of them produce toxic reactions to the patient but also due to the emergence of drugs-resistant bacteria [3,4,5,6,7]. The need for new, effective and affordable drugs to treat infectious diseases in the developing world is one of the major issues facing global health today.

Antibacterial drugs derived from natural source play significant roles in the prevention and treatment of human infection diseases [1,8,9]. Natural bioactive compounds would be best solution to overcome the effects of antibiotics resistance bacteria with no or less side effects [10,11]. In recent

decades, many studies have been carried out on different plant species to discover compounds of possible interest for medicinal application against bacterial infections [12,13,14]. Among these studies, several have focused on the biological and phytochemical properties of different species of mangrove [15,16,17].

There are about 3.6 million hectares of mangrove forests which grew along 95,000 km of Indonesian coastline and it represent approximately 23% of the total mangrove forests in the world [18,19]. *Brugutera cylindrica* is one of the many mangroves species which spread along Indonesian coastline. However, the antibacterial potentials of this Indonesian mangroves are yet to be discovered.

Another factor that renewed the exploration of mangrove is the rapid rate of plant species extinction in the past 20 years and the most threatened plant species are found in the tropics [20]. Mangrove forests suffered a shrinkage of about 1-2% every year [21]. Before any medicinal use or other important features can be assessed, it is likely that some species will extinct before they are even discovered.

The aim of this study was to explore the antibacterial activity potency of *B. cylindrica* leaf extracts against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

2. Methods

2.1. Plant Materials

The leaves of *B. cylindrica* harvested from Kawasan Ekowisata Mangrove, Pantai Indah Kapuk, North Jakarta, Indonesia with the permission from Department of Marine, Agriculture, and Food Security Special Capital Region of Jakarta. *B. cylindrica* was identified by Indonesian Institute of Sciences (LIPI) taxonomist at Cibinong Science Center. Before used, the leaves were firstly cleaned with tap water to remove dirt and then rinsed with distilled water. The leaves were cut into transverse of approximately 2 cm width and dried by using oven (Memmert, Germany) at 45 °C for 12 hours. The dried leaves were ground into powder using blender (Cucina Philips, Indonesia) and screened by using mesh number 35 (CISA Cedaceria Industrial, Spain). The powder was then transferred into glass bottles with rubber stopper, wrapped in aluminium foil and stored in the freezer at -20 °C prior to extraction.

2.2. Extraction

The method was based on method described by Dhayanithi et al. [22] with slightly modification. The leaf powder transferred into an erlenmeyer flask and immersed in solvent (1:10) for 24 hours in a shaker set at 125 rpm. The erlenmeyer flask mouth was sealed with aluminium foil and Parafilm and the whole flask was covered with aluminium foil to protect the content from light. After 24 hours maceration, the extracts were filtrated through cotton fine meshed cloth. The filtrates were then centrifuged (Hettich Rotina 35R centrifuge, Germany) for 10 minutes at 10,000 rpm, 25 °C. The supernatants were then collected by using graduated pipette and prepared for further phytochemical analysis.

2.3. Determination of total phenolic content

The determination of total phenolic content (TPC) of extracts obtained was adapted from Banerjee et al., 2008 [23] with slightly modification and calibrated against galic acid as the reference standard. A 0.3 ml sample was mixed with 1.5 ml of Folin-Ciocalteu reagent and 1.2 ml of sodium carbonate (7.5%), consecutively. The mixture of each step should be mixed well by using vortex (Genie 2 mixer, Scientific Industries, USA) and allowed to stand for one hour in a dark chamber. Absorption was measured by using UV-Vis spectrophotometer (Genesys 10 UV-Vis spectrophotometer, Thermo Electron Corporation, USA) at 765 nm. The standard curve galic acid was prepared by diluting the stock standard with the extraction solvents to yield 50 to 200 ppm TPC. The results were calculated according to the calibration curve for galic acid and crude extract of TPC derives from quadruplicate analyses and expressed as galic acid equivalents (GAE mg/ g) of dry material (DM).

2.4. Determination of flavonoid content

The determination of flavonoid content (FC) was adapted from Do et al. [24] with slightly modification and calibrated against quercetin as the reference standard. The Leaf powder was first diluted with its solvent to reach dilution factor of 25 for test the FC in the samples. 1.5 ml of methanol in test tube was prepared. A 0.5 ml sample was mixed with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water, consecutively. The mixture of each step should be mixed well by using vortex. The mixtures were incubated in a dark chamber for 30 minutes and absorbance was measured by using a UV-Vis spectrophotometer at 415 nm. The standard curve quercetin was prepared by diluting the stock standard with the extraction solvents to yield 20 - 200 ppm of FC. The results were calculated according to the calibration curve for quercetin and crude extract of FC derives from quadruplicate analyses and expressed as quercetin equivalents (QE mg/ g) of dry material (DM).

2.5. Antibacterial activity

The study of antibacterial activity was conducted in two conditions, the wet extracts study which held at Swiss German University and conducted by using three different solvents (water, ethanol, and n-Hexane) against two types of bacteria, *E. coli* and *S. aureus*. While the dried extracts study which held at Biopharmaca Tropica Research Center, LPPM IPB and conducted by using four different solvents based on the level of polarity (water, ethanol, chloroform and n-Hexane), against three types of bacteria, *E. coli*, *S. epidermidis* and *S. aureus*.

The Agar disk diffusion assay has been widely used to assay plant extract for antibacterial activity [25]. *E. coli*, *S. epidermidis* and *S. aureus* were adjusted to certain concentration, 1 x 10^8 CFU/ml or 0.5 mcFarland's standard [26], were inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. In this method, 6 mm sterilized filter papers disks (Whatmann No. 1) were soaked into *B. cylindrica* extract and air dried for one hour at bio safety cabinet. Each type of extracts replicated by three. The paper disks which was impregnated with diluted extracts were placed on the surface of each MHA plate by using a sterile pair of forceps/ pincers. Then the plates were incubated at 37°C for 24 hours and diameter of the zone of inhibition (ZOI) obtained was measured by a ruler or caliper. The bigger diameter of the ZOI, the more susceptible of the microorganism to the extracts.

2.6. Statistical analysis

Data result was analyzed by Analysis of Variance (ANOVA) single factor. The P-two tail or P-Value below 0.05 ($P = 0.05$) indicated that the data were significantly different, while if P-Value above 0.05 ($P > 0.05$), the data were insignificantly different. The data graphic was built using Microsoft Excel 2010.

3. Results and Discussion

Extraction of plant materials depends on various factors such as solvents, methods, and extraction time to separate different quality and quantity of bioactive components in the crude extracts [27]. Polarity of solvent plays key role in the outcome of bioactive compound extracted from *B. cylindrica*. The wet extracts with ethanol showed the highest total phenolic content compared with water and n-Hexane, consecutively (Figure 1). It was because ethanol has the ability to bind with hydrophilic compounds such as flavonoid and phenolic compound [28]. Ethanol is chosen when water-insoluble constituents need to be extracted. However, higher ethanol percentages do not necessarily mean higher extraction activity [29].

The wet extracts with ethanol also showed the highest flavonoid content compared with water and n-hexane, consecutively (Figure 2). Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are best extracted with chloroform, dichloromethane, diethyl ether, or ethyl acetate, while flavonoid glycosides and more polar aglycones are best extracted with alcohols or

alcohol–water mixtures [30,31]. The combination of water and organic solvent facilitate the extraction of chemicals that are soluble in water and/or organic solvent [24].

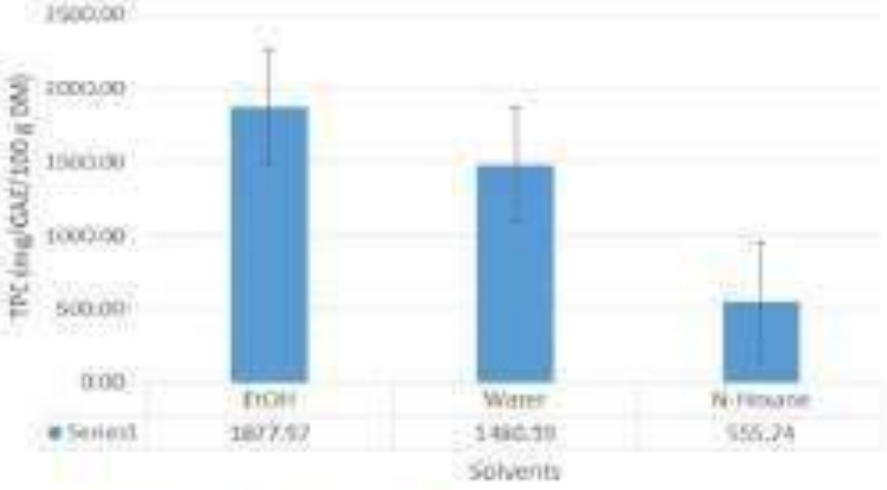

Figure 1. Total phenolic content of *B. cylindrica* leaf extracts

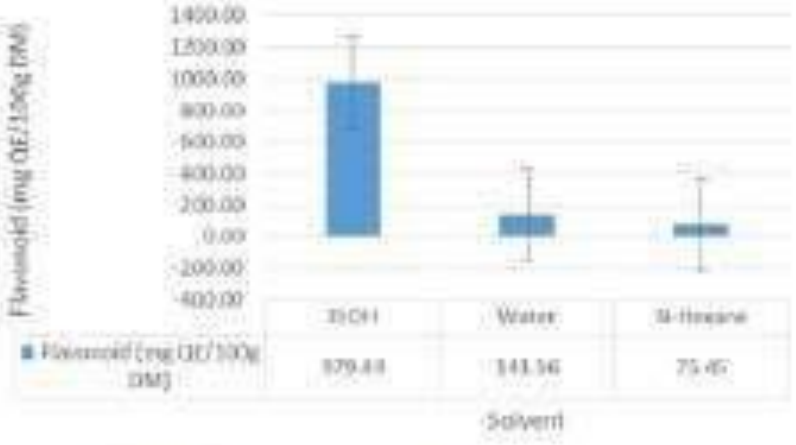

Figure 2. Flavonoid content of *B. cylindrica* leaf extracts

The mechanisms for phenolic toxicity to microorganisms include adsorption and disruption of microbial membranes, interaction with enzymes, and metal ion deprivation [32,33,34]. The higher FC was also expected to show the higher antibacterial activity since flavonoids are synthesized by plants in response to microbial infection [35,36,37,38]. Flavonoid involved in inhibition nucleic acid biosynthesis, inactivate microbial adhesins enzymes and cell envelope transport proteins [39,40]. Lipophilic flavonoids may also disrupt microbial membranes [13,36,41].

The ZOI obtained of the wet extracts with ethanol against *S. aureus* and *E. coli* were 14.30 and 13.30 mm, respectively (Figure 3). The dried extracts study also showed a consistent results with the wet extracts study. The ZOI of the dried extracts with ethanol against *S. aureus*, *E. coli*, and *S. epidermidis* were 9.32, 6.20 and 6.59 mm, as showed in Table 1. Taken altogether, the leaf extracts can be categorized as bacteriostatic due to the capability of bacterial growth inhibition. Quantitatively, the leaf extract can be classified as weak-to-moderate antibacterial since the ZOI obtained around 10

to 15 mm. While, the ZOI obtained by Ciprofloxacin, tetracycline and chloramphenicol were 41.2, 22.10 and 20.80 mm.

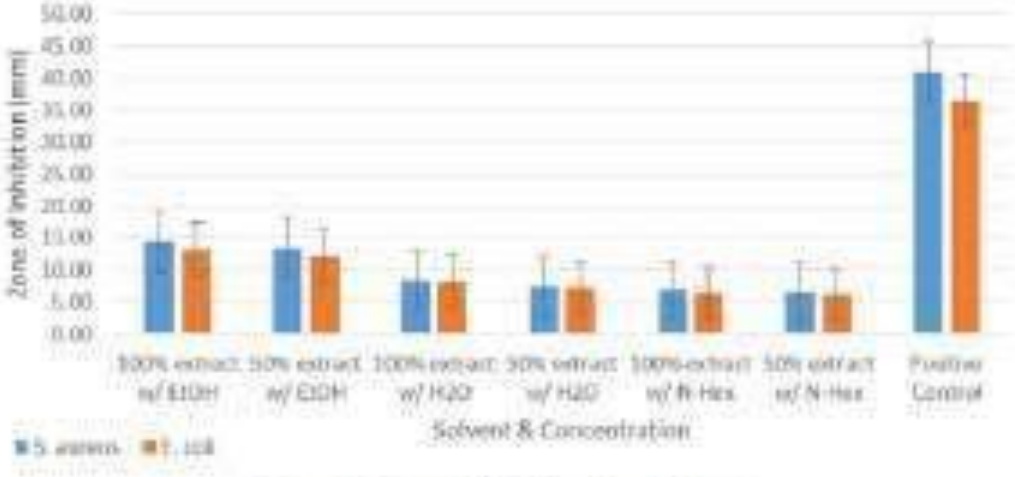

Figure 3. Zone of inhibition of wet extracts

Table 1. Antibacterial test of *B. cylindrica* leaf extracts against *E. coli* and *S. aureus*

Conc	(+) mm		Extraction						(-) mm
(%)	Tetra	Chloram	b. Cyl ethx (mm)			b. Cyl chlx (mm)			DMSO
	22.10	20.80	SA	EC	SE	SA	EC	SE	
0.4			9.32	6.20	6.59	7.32	6.88	7.42	
0.2			8.32	6.00	6.32	7.00	6.52	6.26	
0.1			8.08	6.00	6.16	6.72	6.00	6.00	
0.05			6.40	6.00	6.20	6.56	6.02	6.52	
									6.00

Conc : concentration, tetra : tetracycline, chlor : chloramphenycol, b. cyl ethx : *B. cylindrica* ethanol extract, b. cyl chlx : *B. cylindrica* chloroform extract, r. muc ethx : *R. mucronata* ethanol extract, r. muc chlx : *R. mucronata* chloroform extract, DMSO = DMSO 20%, SA: *S. aureus*, EC: *E. coli*, and SE: *S. epidermidis*.

Since all the extracts consist of many different compounds, they presumably contain single compounds with higher activity than the value obtained as crude extract. In addition, isolation and characterization of the antibacterial compounds presented in these extracts seemed to be worthwhile [42]. The potential for developing antimicrobial agents from mangrove species appears rewarding, as it may lead to development of phytomedicine against pathogenic microbes [43].

Study with different sampling time, age of plant, plant part, solvent, method of extraction and time required for extraction would be necessary [44,45]. Because, many of the phytochemicals in plants can be detected at different concentrations because the amount and composition of secondary metabolites are not constant and their concentration depends on the tissue type and the age of the plant [46,47].

In general, both type of extracts showed significant antibacterial activity against gram-positive bacteria. This matter may be correlated with the cell envelopes composition. The cell envelopes of most bacteria fall into one of two major groups. Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker than is found in the Gram-negatives [48]. Therefore, gram-negative bacteria is more resistant than gram-positive due to the lipopolyproteins and peptidoglycan

composition in their cell envelope [49]. Gram-negative cell walls is strong to withstand three atm of turgor pressure, tough to endure extreme temperatures, pH and elastic to be capable of expanding several times their normal surface area [50].

Conclusions

The study of *B. cylindrica* leaf extract suggested that ethanol was the best solvent. The widest area of ZOI was showed by wet extracts with ethanol against *S. aureus* and *E. coli* were 14.30 and 13.30 mm, respectively. While, the ZOI dried extracts with ethanol against *S. aureus*, *S. epidermidis* and *E. coli* were 9.32, 6.59 and 6.20 mm, respectively. In conclusion, both type of extracts showed considerably high antibacterial activity against gram-positive bacteria as crude extracts.

Acknowledgments

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