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Lampiran 25. Submitted Paper on IOP Conference Series: Earth and Environmental Science, ''Introduction of bioprospecting opportunities for Indonesian mangrove species'', International Biotechnology Conference on Estate Crops 2017, 18- 20 October 2017 in Bali, Indonesia.

Introduction of bioprospecting opportunities for Indonesian mangrove species

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

1. Introduction

Indonesia has a large number of plant biodiversity, approximately 30,000 – 50,000 species of plants, but only 7500 species of them known to be efficacious as drugs ingredient [1]. In term of mangrove biodiversity, Indonesia represents approximately 22.6% of the total mangrove ecosystems in the world [2]. Therefore, Indonesia becomes the country with the world's largest mangrove forest [3]. According to Romimohtarto and Juwana [4], there are at least 202 mangrove plant species recorded in Indonesia, including 89 types of tree, 5 types of Palma, 19 types of scandent, 44 types of herbaceous ground, 44 types of epiphyte and one type of fern. Some types of Indonesian mangrove are *Avicennia*, *Sonneratia*, *Rhizophora*, *Ceriops*, *Bruguiera*, *Lumnitzera*, *Excoecaria*, *Xylocarpus*, *Aegiceras*, *Scyphyphora*, and *Nypa* [5].

Mangrove is a plant that well adapted to many ecological stresses by alterations not only their morphology but also physiological processes resulting in the synthesis of novel chemical compounds that offer protection to these plants against various biotic and abiotic stresses. So, it should not be surprising that there are a large number of different chemical properties that exist for different

functions [6]. A number of mangrove's secondary metabolites have significant pharmacological properties that have been used traditionally for the treatment of diseases [7]. Mangroves are a very potential as drugs source among a great number of wild medicinal plants biodiversity.

The prospective study of biodiversity looking for applications in agriculture, industry and medicine is known as bioprospecting. However, the best known is in the pharmaceutical industry. In this way, natural products have important contributions in drug discovery as acetylsalicylic acid (ASA) (painkiller derived from willow bark, *Salix alba*), reserpine (antihypertensive from *Rauwolfia serpentina*, known as Indian snake root), d-tubocurarine (muscle relaxant used in anesthesia from *Chondrodendron tomentosum*), artemisinin (derived from *Artemisia annua* used as an anti-malarial agent), vincristine and vinblastine (anti-cancer drugs derived from *Catharanthus roseus* [8]. Therefore, mangrove plants with numerous bioactive compounds should be suitable for bioprospecting program.

The aim of this article is to provide a rational approach to design a bioprospecting program as an initiation on the primary screening of novel drugs from Indonesian mangrove species as a following up of extract library establishment of Indonesian mangrove initiated by Audah *et al.* [9]. Hence, mangrove forests suffered a shrinkage of about 1-2% every year globally [10]. If the biodiversity is not properly managed, it will soon only become a history.

2. Materials and Methods

Bioprospecting can be achieved through information management and inventory of biodiversity, basic research, applied research, product development, production, marketing, and distribution. But in this article only a few steps will be discussed, among others:

2.1. Information management and inventory of mangrove diversity

The approach in the sample collection can be based on its use as a traditional medicine and may also be randomized. The nationally programmed collection will make it easier for research related to the conservation and understanding of its genetic resources. Identification of the species taxonomy is very important both to increase the probability of finding new species containing new compounds and avoiding known compounds.

The collection process should take into account the concept of species conservation and the habitat of collection subjects. Because research on target compounds will require more material in progressive amounts and will ultimately have an impact on the environment. Therefore, it must be ensured that the initial collection is well documented. To ensure the same samples the prior sampling process must be equipped with GPS data, complete documentation and good taxonomic knowledge [11].

2.2. Development of extraction method

Extraction must ensure that all extracts are available for screening, isolation, and elucidation of chemical structures. Degradation of compounds can occur in extracts stored for long periods of time. The natural product can be stored as simplicial which can maintain the integrity of the compound. However, humidity should be controlled to prevent damaged samples; high humidity increases the chances of fungi and microorganisms growing. Samples should be stored in each particular container and given a barcode.

2.3. Screening of antimicrobial and anticancer activity

Drugs discovery derived from plants has been the focus of world researchers since they have the potential to counter the threat of resistant bacteria and have anticancer properties. Several *in vitro* methods may be used to evaluate the antibacterial activity of extracts or pure compounds. The most well-known basic methods are the disc diffusion and broth dilution method. The screening process for potential anti-cancer natural ingredients can be performed by Brine Shrimp Lethality Test (BSLT). BSLT is considered quite economical and uses a small amount of test material. Since its introduction, *in vivo* tests have been shown to be represented as a cytotoxic active fractionation bioassay guide and anticancer agents [12].

2.4. Fractionation, isolation of pure compounds and establishment of extract library

When the extract exhibits biological activity, fractionation is necessary to separate groups of compounds with similar chemical-physical properties, such as solubility and acidity [13]. Each fraction was tested and the active sample had to be fractionated repeatedly to increase the purity of the compound. The process of testing and fractionation is carried out continuously until a pure compound is obtained which is responsible for certain biological activities. However, the repeated fractionation process needs to be prevented by the process of dereplication [14].

The extract library is a collection of active compound extracts from natural ingredients used for screening biological targets. A quality extract library will be a way of discovering natural materials that can be developed into drugs and become the identification of the starting point of chemical drug optimization.

2.5. The establishment of extract library consortium

The establishment of *extract library consortium* was initiated with the aim that the research activities of medicines from natural materials, especially mangroves, which have been done separately by each researcher, can be better organized, synergize and avoid overlap with each other.

3. Results and Discussion

The crude extracts obtained from the study were 64 extracts. The extraction was conducted by a re-maceration method with four different solvents (hexane, ethyl acetate, ethanol and water). In general, most extracts is effective against gram-positive bacteria and have potential to be an anticancer agent. Mangrove plants that used in the research are 8 species out of more than 20 species, so there are more bioactive compounds that have not been explored since different species might contain different bioactive compounds.

The crude extracts that are shown to have further activity can be fractionated and isolated to obtain a single compound which can be developed into a natural medicine ingredient. Furthermore, 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 can be performed to ensure the desired safety and efficacy. In order to compete in the world market, in addition to product quality there is also a very decisive factor, namely the genetic resources of products of interest to the world market and have access to quality databases of various commodities that become a market demand.

Qualitative phytochemical analysis showed that mangrove leaf extract of *Rhizophora stylosa* and *Avicennia marina* contained tannins, flavonoids, terpenoids, alkaloids, flavonoids, and phenolic glycosides [15]. Mangrove extract also contains several bioactive compounds such as flavonoids, saponins, tannins, and triterpenoids [16]. As far as the author's observation, mangrove utilization in Indonesia is limited to conservation only. Potential mangroves as ingredients of the drug received less attention. Therefore, bioprospecting programs for mangrove plants need to be initiated.

Essentially bioprospecting has two basic goals, among others (1) sustainable use of genetic resources and conservation, and (2) socio-economic development for a country rich in biodiversity. This modern bio-prospecting concept gives developing countries a way to improve their national capacity to add value to natural resources, build skills, infrastructure and technology to develop new products for global markets while ensuring the protection and use of natural resources that is sustainable [6]. In conducting biodiscovery research, it should be noted that if large numbers of re-collections may not be possible, so the target compounds should be chemically synthesized.

Bioprospecting is constituted as scientific and economic activities with a high impact on sustainable development and poverty reduction as well as economic growth for developing countries. Because the establishment biodiversity-based industries produce social appropriation of knowledge showing the benefits and applications of biodiversity [6]. Also, the revenue from royalties represented by the development of new medicines (have been estimated that a successful drug can generate $1

billion per year) derived from natural sources contribute to the conservation of biodiversity in mega diverse countries as well as the protection of traditional medical knowledge [8].

The main parameters to be considered in bioprospecting are the most energy and capital investment needs in the technological and product development process. For example, in the search for drugs, the most complex part is basic research of disease and potential clinical testing of drugs. The main screening of compounds is usually cheap and technically easy to do. A country wishing to promote national capacity-building in drug development can follow a capability-enhancing model that begins with a major screening for obtaining compounds and access to technology in screening, while testing and clinical trials are at an early stage [8].

4. Conclusions

Mangrove plants are potential for bioprospecting program. Bioprospecting should be based on sustainable use of biodiversity. 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 socio-economic development, human resource development, science and technology capability, market analysis, sustainable capital, and strategic plan should be developed.

5. Acknowledgements

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Lampiran 26. Dokumentasi sebagai pembicara dan Penandatanganan Perjanjian Kerjasama Pembentukan Konsorsium Indonesia untuk Extract Library Bahan Alam pada acara Seminar Nasional Herbal di SGU pada 26 April 2018.

Lampiran 27. Surat undangan sebagai invited Speaker pada Computer Aided Drug Design (CADD) 2018 Seminar & Workshop, Bali, 26-30 November 2018.

Bandung, 25th June 2018

No : 12/CADD/LOI/VI/2018
Subject : Invitation as Speaker for the Forthcoming CADD 2018

Dr. Kholis A. Audah
Swiss German University

Dear **Dr. Kholis A. Audah**

On behalf of the Committee of Computer Aided Drug Design (CADD) 2018 Seminar & Workshop, we cordially invite you as a speaker in CADD 2018, which will be held from November 26th- 30th, 2018 in Kuta - Bali, Indonesia. Our Seminar theme would be **"The New Era of Digital Approach in Drug Design"**. You can see our website at http://farmasi.unpad.ac.id/cadd2018/ .

We would like to apologize that due to our limited budget, we could not support any national or international travel fares, however, in recognition to your contribution, we shall waive the registration fees and provide full hospitality during the seminar.

We would be grateful if you could confirm your acceptance. We would also greatly appreciate receiving the title(s) and abstract(s) of your proposed talk(s) before September 3rd 2018. Please do not hesitate to contact us for any additional information.

Thank you very much and looking forward to seeing you in Bali.

With kindest regards,

Dr. Sandra Megantara
Vice Chairman of CADD 2018

Lampiran 28. Perjanjian Kerjasama Konsorsium Indonesia untuk Extract Library Bahan Alam

PERJANJIAN KERJASAMA
KONSORSIUM INDONESIA UNTUK EXTRACT LIBRARY BAHAN ALAM
(INDONESIAN CONSORTIUM ON NATURAL PRODUCT EXTRACT LIBRARY)

Academic Research and Community Services Universitas Swiss German
Lembanga Penelitian dan Pengabdian kepada Masyarakat cq Tropical Biopharmaca Research Center
Institut Pertanian Bogor
Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia
Fakultas Kedokteran Universitas Lampung
Fakultas Ilmu Kesehatan Universitas Esa Unggul

Nomor: SPK/ARCS/002/IV/2018 (ARCS Swiss German University)
Nomor: 2229/IT3.L1/KS/2018 (LPPM IPB cq Tropical Biopharmaca Research Center)
Nomor: B-576/IPT.2/KS.02/IV/2018 (Pusat Penelitian Kimia LIPI)
Nomor: 143/UN.26.8/KS/2018 (FK UNILA)
Nomor: 124/FIKES/DKN/IV/2018 (FIKES Universitas Esa Unggul)

Pada hari ini, hari Kamis, tanggal 26 bulan April tahun 2018, telah dibuat perjanjian kerjasama pembentukan Konsorsium Indonesia untuk Extract Library Bahan Alam (untuk selanjutnya disebut Perjanjian) yang terdiri dari:

A. Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Swiss German, berkedudukan di Prominence Tower, Jalan Jalur Sutera Barat Kav. 15, Alam Sutera, Tangerang, Banten 15143

B. Lembaga Penelitian dan Pengabdian kepada Masyarakat cq Tropical Biopharmaca Research Center Institut Pertanian Bogor, berkedudukan di Kampus IPB Taman Kencana Jl Taman Kencana No 3, Bogor 16128

C. Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia, berkedudukan di Gd. 452 Kawasan PUSPIPTEK Serpong, Tangerang Selatan, Banten 15314

D. Fakultas Kedokteran Universitas Lampung, berkedudukan di No. 01, Jl. Prof. Dr. Ir. Sumantri Brojonegoro, Kp. Baru, Kedaton, Kota Bandar Lampung, Lampung 35141

E. Fakultas Ilmu Kesehatan Universitas Esa Unggul, berkedudukan di Jl. Arjuna Utara No.9 Kebon Jeruk West Jakarta 11510

Selanjutnya semua PIHAK di atas disebut ANGGOTA KONSORSIUM. ANGGOTA KONSORSIUM sepakat untuk mengadakan kerjasama membentuk '**Konsorsium Indonesia untuk Extract Library Bahan Alam**' dengan ketentuan sebagaimana tertera di bawah ini.

PASAL 1
BENTUK KERJASAMA

(1) ANGGOTA KONSORSIUM dengan ini mengikatkan diri untuk bekerjasama dalam pembentukan Konsorsium Indonesia untuk Extract Library Bahan Alam.

(2) Bahwa kegiatan-kegiatan yang dilaksanakan dalam kerjasama antara ANGGOTA KONSORSIUM direncanakan dan dilakukan bersama sesuai dengan kapasitas masing-masing dan saling menguntungkan secara proporsional.

PASAL 2
KEWAJIBAN PARA PIHAK

(1) Berperan aktif dalam penyiapan Extract Library Bahan Alam sesuai dengan tugas pokok dan fungsi masing-masing ANGGOTA KONSORSIUM dan akan dituangkan dalam Rencana Kerja sebagai bagian yang tidak terpisahkan dari perjanjian kerjasama ini.

1 / 5

PASAL 12
LAIN-LAIN

Segala perubahan dan hal-hal lain yang belum atau tidak cukup diatur dalam kesepakatan ini, akan diatur lebih lanjut oleh PARA PIHAK dalam kesepakatan tambahan (addendum) sebagai bagian yang tidak terpisahkan dengan kesepakatan ini.

PASAL 13
PENUTUP

Kesepakatan ini dibuat dan ditandatangani di Tangerang oleh PARA PIHAK dalam rangkap enam, bermaterai cukup serta mempunyai kekuatan hukum yang sama.

Tangerang, 26 April 2018

Lembaga Penelitian dan Pengabdian Masyarakat Universitas Swiss German
(Direktur)

(Dr. Ing. Evita H. Legowo)

Lembaga Penelitian dan Pengabdian kepada Masyarakat Institut Pertanian Bogor
(Kepala)

(Dr. Ir. Aji Hermawan, M.M)

Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia
(Kepala)

(Dr. Eng Agus Haryono)

Fakultas Kedokteran Universitas Lampung
(Dekan)

(Dr. dr. Muhartono, M.Kes, Sp.PA)

Fakultas Ilmu Kesehatan Universitas Esa Unggul
(Dekan)

(Dr. Aprilita Rina Yanti Eff, Apt, M.Biomed)

Lampiran 29. MoA antara UMS Sabah dengan SGU untuk kerjasama riset Uji anti Tuberculosis dan Demam Berdarah Dengue Mangrove Extract Library

IN WITNESS WHEREOF UMS and **SGU** hereunto have executed this Memorandum of Agreement on the year and date first above written:

SIGNED BY
for and on behalf of

UNIVERSITI MALAYSIA SABAH

PROF. DATUK DR. D. KAMARUDIN D MUDIN
Vice-Chancellor

SIGNED BY
for and on behalf of

SWISS GERMAN UNIVERISTY

DR. RER. NAT. FILIANA SANTOSO
Rector

In the presence of:

NO'MAN HJ. AHMAD
Registrar

In the presence of:

DR. KHOLIS M AUDAH
Principal Investigators

Lampiran 30. Surat undangan sebagai pembicara pada acara Seminar on Development and Application of Indonesian Herbal Products, SGU, 26 april 2018.

From: Diah Indriani Widiputri <diah.widiputri@sgu.ac.id>
Date: 26 March 2018 15.53.00 GMT+7
To: audahka@gmail.com
Cc: Hery Sutanto <hery.sutanto@sgu.ac.id>, Silvya Yusri <silvya.yusri@sgu.ac.id>
Subject: Konfirmasi Pembicara - Kholis A. Audah

Yang terhormat Bapak Kholis A. Audah, M. Sc, Ph.D.,

Sehubungan dengan persetujuan Bapak untuk menjadi Pembicara pada acara Seminar Herbal dengan judul "Seminar on Development and Application of Indonesian Herbal Products" di Swiss German University (SGU) pada hari Kamis, 26 April 2018, dengan ini kami bermaksud menyampaikan beberapa informasi teknis yang berkaitan dengan sesi presentasi Bapak, sebagai berikut:

1. Mohon dapat mengisi dan mengirimkan kembali kepada kami Formulir Konfirmasi Pembicara yang terdapat pada lampiran email ini. Kami mohon agar dapat mengirimkan formulir tersebut selambat-lambatnya pada hari Senin, 2 April 2018.

2. Sesi presentasi Bapak memiliki topik: "Development of Indonesian Herbal Products". Mohon agar dapat menyiapkan materi yang terkait dengan topik tersebut dalam Bahasa Indonesia.

3. Waktu presentasi yang tersedia adalah 30 menit (diluar sesi tanya jawab).

4. Sesi tanya jawab akan dilakukan dalam bentuk Panel Discussion untuk 3 Pembicara, pada akhir tiap sesi.

5. Target peserta yang diundang dan diharapkan untuk hadir berasal dari kalangan praktisi industri herbal, UMKM Herbal, institut penelitian, perwakilan universitas, dan guru.

Demikian informasi yang dapat kami sampaikan. Jika Bapak memerlukan informasi lebih lanjut, mohon menghubungi kami, baik melalui email ataupun nomor telepon: 0815 912 6446 (Diah).

Hormat kami,

Panitia Penyelenggara
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Lampiran 31. Surat Undangan sebagai Penguji Ujian Kolokium di FMIPA-IPB

KEMENTERIAN RISET, TEKNOLOGI, DAN PENDIDIKAN TINGGI
DEPARTEMEN KIMIA
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1 November 2018

NOTA DINAS
Hal : Undangan kolokium

Yth Dr.Irmanida Batubara, SSi, MSi
Dr. Kholis Abdurachim Audah, MSc
Prof.Dr.Dra. Purwantiningsih, MS
ditempat

Dengan ini kami mengharapkan kesedian Bapak/Ibu untuk menjadi penguji **Ujian** Kolokium:

Hari : Jumat
Tanggal : 2 November 2018
Waktu : 08.00-09.00 WIB
Tempat : Ruang Sidang Departemen Kimia

Pemberi kolokium adalah Saudara **Angga Crytal Loasana NIM G451170301** dengan judul: **Penapisan dan Penentuan Senyawa Antioksidan dan Antibakteri Patogen (*Vibrio Cholerae*) Batang *Bruguiera gymnorrhiza*.**

Pembimbing Utama : Dr.Irmanida Batubara, SSi, MSi
Pembimbing Anggota : Dr.Kholis Abdurachim Audah, MSc

Besar harapan kami, Bapak/Ibu dapat hadir tepat pada waktunya. Terima kasih

Sekretaris Program Studi Magister,

Dr. Wulan Tri Wahyuni, SSi, MSi
NIP198211232012122002

Tembusan Yth:
Bendahara Dept Kimia

Lampiran 32. Surat Undangan Pertemuan PERHIPBA 2018

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
INSTITUT PERTANIAN BOGOR
LEMBAGA PENELITIAN & PENGABDIAN KEPADA MASYARAKAT
PUSAT STUDI BIOFARMAKA TROPIKA

Jl. Taman Kencana No. 3, Kampus IPB Taman Kencana, Bogor 16128
Telp 0251-8373561 Faks 0251-8347525 E-mail: bfarmaka@gmail.com

Bogor, 23 Oktober 2018

No : 264/IT3.L1.13/HM/2018
Lampiran : 1 berkas
Hal : Pertemuan PERHIPBA 2018

Kepada
Yth Bapak/Ibu
Daftar Undangan Terlampir
Di Tempat

Dengan hormat,

Perkenalkan Saya, Irmanida Batubara dari Pusat Studi Biofarmaka Tropika LPPM IPB. Saya mendapatkan amanah untuk menjadi pengurus Perhimpunan Peneliti Bahan Obat Alami (PERHIPBA) menggantikan Prof Bambang Prajoyo. Oleh karena itu, saya merasa perlu melakukan koordinasi dan konsolidasi para pengurus dan anggota PERHIPBA. Untuk membuat pertemuan lebih bermakna pada pertemuan tersebut kami berencana mengadakan seminar PERHIPBA yang berkolaborasi dengan Professor Eiichiro Fukasaki (Osaka University) yang merupakan salah satu professor kolaborator Pusat Studi Tropika LPPM-IPB yang datang ke Indonesia dalam rangka *World Class Professor* (WCP) Skema A.
Untuk keperluan seminar tersebut, diharapkan dukungan dan kontribusi Bapak/Ibu untuk dapat menjadi salah satu pembicara dalam kegiatan seminar tersebut. Pelaksanaan seminar akan diadakan,

Hari/Tanggal : Kamis/1 November 2018
Pukul : 08.00 - 16.30 WIB
Tempat : Kampus IPB Darmaga

Mengingat pentingnya kegiatan ini, kehadiran dan kontribusi Bapak/Ibu sangat kami harapkan. Mohon konfirmasi kesediaan menjadi pembicara dan kehadirannya, dapat disampaikan melalui email di: bfarmaka@apps.ipb.ac.id / Whatsapp ke Sdri Disa (081210315643) sebelum tanggal 30 Oktober 2018. Atas perhatian dan kerjasamanya disampaikan terima kasih.

Pusat Studi Biofarmaka Tropika LPPM-IPB

Dr. Irmanida Batubara, SSi, MSi.

F-RM/ADM-11/02/00

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
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Anticancer Screening of Mangrove Extract Library: Accelerating Drug Discovery from Indonesian Biodiversity

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Abstract: Cancer is still one of the leading death causes in the world. There will be an estimated 18.1 million new cancer cases and over 9 million cancer deaths in 2018. Furthermore, some studies reported that new types of cancer had been discovered. Therefore, it is very important to find different sources for anticancer agents. 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 anticancer 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. Brine Shrimp Lethality Test (BSLT) and MTT Assay were conducted for anticancer screening of mangrove extract library. There were 43 extracts showed anticancer potential with LC_{50} < 1000 ppm and 21 extracts with LC_{50} >1000 ppm. Next, 16 out of 43 extracts which have LC_{50}< 500 ppm and some extracts with LC_{50} >1000 ppm were selected to be evaluated in MTT Cytotoxicity assay. There were 3 extracts that highly inhibited HeLa and MCF-7 Cell line. The highest inhibition percentage was recorded for ethyl acetate extract of root of *Avicennia Marina* (85 Ea), although it was not toxic enough in BSLT due to the high LC_{50} value (>1000 ppm). The next highest inhibition percentage were recorded for water extract of leaves of *Sonneratia alba* (88 A) and ethyl acetate extract of leaves of *Xylocarpus granatum* (86 Ea). In addition, there were 2 extracts that only effective to inhibit HeLa cell line, namely ethanol extract of leaves *Bruguiera gymnorrhiza* (75 Et) and ethyl acetate extract of root of *Rhizophora apiculata* (74 Ea). Phytochemical analysis of the extracts was also evaluated. The majority of samples showed saponin and tannin in considerable amount. This supported the data that mangrove extracts were potential as anticancer agents.

Keywords: Anticancer, drug discovery, drug resistant, extract library, Indonesian biodiversity.

1. Introduction

Cancer is still one of the leading death causes in the world. There will be an estimated 18.1 million new cancer cases and over 9 million cancer deaths in 2018 (Bray *et al.*, 2018). Lung cancer is the most frequently diagnosed type of cancer and become the leading cause of cancer deaths in male and female.

On the other hand, effective cancer therapy is still a great challenge for nowadays society due to some limitations and side-effects from the treatment. Therefore, finding alternatives for drug sources as anticancer agent is urgently required. Meanwhile, current anticancer research shows that herbal plants from tropical region such as mangrove have a potential cytotoxicity effects towards cancer cells (Kerry *et al.*, 2018). In addition, anticancer drug discovery is a lengthy and expensive process. Through screening process utilizing natural products, it can become a solution of the slow and expensive drug discovery process using conventional way. Indonesia is well known as one of the richest countries in the world in biodiversity where is a home of approximately 11% or more than 30,000 of the world's flowering plants and other biota both in land and marine with significant figures (Rintelen, Arida, & Häuser, 2017). 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 (Bandaranayake, 2002). Along roughly 90,000 kilometers coastline,

Indonesia is home of about 20 families with hundreds of species of mangroves and their associates. Indonesia has the largest mangrove forest or about 23% of total world mangrove forests (Giri *et al.*, 2011). The aim of this study was to evaluate the potential of mangrove extracts (extracts library) as anticancer 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.

2. Research Methods

2.1. Material

The main raw material used in this study was various parts of mangroves plants such as leaves, stem and roots. There were eight different species of mangroves collected from the Eastern Coastline of Lampung Province, Indonesia in May 2017. The species identification was done at the Herbarium of the Center for Biological Research of the Indonesian Institute of Sciences, Bogor, Indonesia. Samples were collected from each parts of the plants in the total of 16 samples.

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

Name of Plants	Part of Plants	Samples Code
zophora apiculata	Root	74
uiera gymnorrhiza	Leaf	75
ophora mucronata	Leaf	79
	Stem	80
vicennia marina	Leaf	84
	Root	85
ocarpus granatum	Leaf	86
Ceriops tagal	Leaf	87
neratia caseolaris	Leaf	88

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

The chemicals and solvents used in this study were obtained from Brata Chem including: Tween-80, Distilled water, ethanol, n-hexane, ethyl acetate 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% (Sigma-Aldrich). For cell culture medium, RPMI medium was used to culture HeLa and MCF-7 cell line supplemented with Fetal Bovine Serum (FBS) and antibiotic-antimycotic.

2.2. Equipment

Equipment used in this study were moisture balance (Mettler Toledo^{TM}), heating and drying Oven (Memmert), Thin-Layer-Chromatography Chamber, ELISA Microplate reader, Bio-Safety Cabinet level 2, cell culture CO_2 incubator, and serological pipette.

2.3. Analytical Procedure

2.3.1. Sample Preparation and Extraction

Preparation of sample and extraction were performed based on previous study (Audah *et al*., 2018). Sample preparation was carried out using an oven with a temperature of less than 50 ºC 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. Extraction method used was gradient maceration (Farida *et al.*, 2014). Four different solvents were used, which were *n*-hexane, ethyl acetate, ethanol and water. yields were then determined based on the ratio of concentrated extract weight with initial sample weight. Extractions were performed triplicate.

2.3.2. 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 105ºC 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).

2.3.3. Qualitative Phytochemical Analysis

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

2.3.4. Brine Shrimp Lethality Test (BSLT)

A tip of eggshell's *Artemia salina* (50–100 mg) was put into a container that had been filled previously with seawater and had been aerated using an aerator. The eggs were hatched at a temperature range of 25–30° C with light exposure until it was ready for use which was 48 hours old. Solution of extract for the test was made with various concentrations: 1000, 500, 100, and 50 ppm then dissolved in sea water with a little addition of Tween-80. Larvae were inserted into the vial (10 larvae/vial) and incubated for 24 hours. The number of dead larvae was quantified in 3 repetitions, then the obtained data were processed using Probit analysis on SPSS version 16 software to obtain LC_{50} value.

2.3.5. MTT Cytotoxicity Assay

MCF-7 and Hela cells line were cultured on 96-well plates in the media of Rosewell Park Memorial Institute (RPMI) with 100 µL medium per well containing approximately 5,000 cells per well. A total of 100 µL of active extract with a concentration of 500 ppm was added to the cultured cells, incubated for 48 hours in a 5% CO_2 incubator at 37° C. Next, 100 µL of tetrazolium monoculture salt (MTT) were added and incubated again for 4 hours in 5% CO_2 incubator. Cells that are still alive will react with MTT to form formazan which can be seen violet. The formed Formazan was dissolved in 96% ethanol. The absorbance reading was in a microplate reader ELISA spectrophotometer at $\lambda = 595$ nm. The tests were carried out using 1× RPMI media as a blank, MCF-7 breast cancer cells and HeLa cervical cancer cells as a negative control, and doxorubicin as a positive control.

3. Results and Discussion

The yield of extracts of the 16 samples were between 0.25% (highlighted green) to 21.18% (highlighted yellow) that belonged to ethyl acetate extract of root of *Rhizophora apiculata* and water extract of leaf of *Xylocarpus granatum* respectively as shown in Table 2. The data clearly showed that root and leaf extract using non-polar solvent (*n*-hexane) resulted in lower yield percentage compared to more polar solvents (root samples 74 and 85, leaf samples 79 and 87) (Zahra *et al.,* 2017). This indicated that root sample contains less nonpolar constituents compared to other parts of plants.

Table 2: Yield of extracts (%)*

Sample Code	Yield (%)	Sample Code	Yields (%)	Sample Code	Yield (%)	Sample Code	Yields (%)
74Ea	**0.25**	79Et	2.48	85H	0.5	86Ea	1.79
75A	20.32	79H	1.04	85Ea	0.89	87A	19.58
75Et	7.09	80A	3.72	86A	21.18	87H	2.41
79Ea	2.36	84Et	7.42	86Et	6.94	88A	7.06

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

Water content of sample (Table 3) 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). 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 3: water content determination

Samples	Part	Shrinkage (%)	Water Content (%)	Samples	Part	Shrinkage (%)	Water Content (%)
R. apiculatae	Root	62	7.36	*A. marina*	Leaf	77	9.43
B. gymnorrhiza	Leaf	63	5.34		Root	71	-
S. caseolaris	Leaf	84	7.23	*X. granatum*	Leaf	80	8.98
R. mucronata	Leaf	71	4.78	*C. tagal*	Leaf	82	9.45
	Stem	47	4.85				

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 anticancer screening. The following results of the dried sample phytochemical test were presented in Table 4.

Table 4: Phytochemical qualitative analysis of samples

Sample Code	Alkaloid			Phenolic					
	M	W	D	F	Sa	Ta	T	S	Q
74	-	-	-	+	+++	+++	-	-	-
75	-	-	-	+++	+++	+++	-	++	-
79	-	-	-	+	+++	+++	-	++	-
80	-	-	-	-	+++	+++	-	-	+
84	-	-	-	++	++	++	-	+	-
85	-	-	-	-	+	-	-	+	-
86	-	-	-	-	+++	+	-	+	-
87	+	+	+	+	+	++	-	+	-
88	-	-	-	++	-	+++	-	+	-

Description: M=Mayer, W=Wagner, D=Dragendorf, T=Triterpenoid, S=Steroid, Q=Quinon, F=Flavonoid, Sa= Saponin, Ta= Tanin

Brine Shrimp lethality Test (BSLT) is one of the initial methods to estimate the level of toxicity of a compound based on the lethal concentration value of 50% (LC_{50}). The shrimp larvae have thin skin and a sensitive body. If there are foreign compounds in the environment, the compound will be easily absorbed into the body of the larva diffusion. The toxic environment due to foreign compound will terminate the shrimp larvae. Chemical compounds are potentially bioactive if they have an LC_{50} of less than 1000 ppm. In this study, there were 43 extracts (table 5) which have the potential to have toxic compounds. After obtaining the LC_{50} value, some extracts were then selected which had LC_{50} below 500 ppm (green highlighted) and some extracts which had LC_{50} values more than 1000 ppm (yellow highlighted) for cytotoxicity testing. The lowest LC_{50} was 40.45 ppm (87 H).

Table 5: Brine Shrimp Lethality Test LC_{50}

Sample Code	LC_{50} (ppm)	Sample Code	LC_{50} (ppm)	Sample Code	LC_{50} (ppm)	Sample Code	LC_{50} (ppm)
74Ea	200.78	79Et	498.28	85H	499.23	86Ea	64.02
75A	>1000	79H	160.43	85Ea	>1000	87A	>1000
75Et	291.61	80A	>1000	86A	408.24	87H	40.45
79Ea	224.45	84Et	229.77	86Et	74.87	88A	488.93

The cytotoxicity of 16 extracts selected from the results of toxicity tests showed water extract of *Sonneratia caseolaris* leaf (88A), ethanol acetate extract of *Xylocarpus granatum* leaf (86Ea), and ethanol acetate extract of *Avicennia marina* root (85Ea) had a high percentage of inhibition in both cells. However, ethanol acetate extract of *Avicennia marina* root (85Ea) did not show bioactive potential that was toxic in BSLT because the obtained LC_{50} was above 1000 ppm. Meanwhile, ethanol extract of *Bruguiera gymnorrhiza* leaf (75Et) and ethanol acetate extract of *Rhizophora apiculata* root (74Ea) have a high percentage of inhibition in HeLa cells line, although it was low in MCF-7 cells line.

Table 6: MTT Cytotoxicity Assay

Sample Code	% Inhibition		LC_{50} (ppm)	Sample Code	% Inhibition		LC_{50} (ppm)
	MCF-7 Cells	HeLa Cells			MCF-7 Cells	HeLa Cells	
74Ea	29.57	85.92	200.78	**85Ea**	98.06	94.04	>1000
75A	45.02	52.8	>1000	86A	44.4	64.8	408.24
75Et	16.2	90.79	291.61	86Et	74.87	85.92	351.52
79Ea	2.03	47.92	224.45	**86Ea**	96.65	92.96	64.02
79Et	18.09	51.71	498.28	87A	41.95	68.05	>1000
79H	-22.96	24.46	160.43	87H	52.53	29.06	40.45
80A	38.78	27.98	>1000	**88A**	97.4	92.42	488.93
84Et	48.37	68.5	229.77	Doxorubicin	94	95.76	-
85H	32.12	29.87	499.23	Control Cell	-	-	-

Description: Green= Highly potential, Yellow= slightly potential

The results of phytochemical analysis of *Avicennia marina* root (85Ea) indicated that there were only two active compounds, namely Saponin and Steroid which were detected in low-concentration. This result can explain the high value of LC_{50} (>1000 ppm) of the extract which was not toxic enough for *Artemia salina.* Extracts of plants or animal are classified a cytotoxic when the LC_{50} value <1000 ppm. An extract is considered very toxic when it has a LC_{50} value < 30 ppm, and toxic if it has a LC_{50} value 30-1000 ppm and is considered non-toxic if LC_{50} > 1000 ppm. While pure compound was declared toxic if it has a value of LC_{50} <200 ppm (Meyer *et al.*, 1982). Meanwhile, other extracts that have more than two compounds, were toxic for *Artemia salina.* For example, the extract of *Ceriops Tagal* leaf (87H) showed that there were seven compounds detected in the extract and had the lowest LC_{50} value of all (40.45 ppm). However, further cytotoxicity evaluation of mangrove extract library was needed to achieve a high degree of sensitivity and reliability such as ATP assay, which has interference-free feature (Karakas *et al.,* 2017).

Extracts of mangrove plant are considered to have potent cytotoxic activity against HeLa and MCF-7 cell lines. When compared between the cell lines HeLa cell lines are more suppressed than MCF-7 cell lines in general (Pradesh, 2018). The presence of different bioactive compounds is the reason for its anticancer activity. Such compounds with antioxidant activity can inhibit mutation and cancer because they scavenge free radicals or induce antioxidant enzymes (Karami *et al.*, 2012). For example, saponins that contained in almost all extracts can inhibit the replication of cellular DNA and the proliferation of cancer cells can be prevented (Yildirim and Kutlu, 2015). In addition, Saponins may stimulate the disintegration of the microtubular network or actin filaments of cancer cells, which can lead to further non-apoptotic cell death (Chen *et al.*, 2009).

4. Conclusion

The methods and the results presented in this study suggested that developing extract library is a highly feasible and crucial for development of anticancer drugs discovery. Mangrove extracts are very potential as natural anticancer and antiproliferative agents. This report can be used as stepping stone towards acceleration anticancer drugs discovery.

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RESEARCH ARTICLE

THE EFFECT OF MANGROVE (Rhizophora apiculata) BARK EXTRACT ETHANOL ON HISTOPATHOLOGY PANCREAS OF MALE WHITE RATS SPRAGUE DAWLEY STRAIN EXPOSED TO CIGARETTE SMOKE

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ABSTRACT

Background: Cigarette smoke is free radical that causing some pathological conditions such as inflammation, proteolysis, and oxidative stress. In previous studies, mangrove (Rhizophora apiculata) bark extract showed the potential effect as an antioxidant. Mangrove and mangrove associates are abundant plants that grow throughout Indonesian shorelines that have high pharmaceutical and food values.

Objective: The purpose of this study is to explore the potential of Rhizophora apiculata bark extract in protecting pancreas of male white rat (Rattus novergicus) Sprague Dawley strain exposed to cigarette smoke.

Methods: This research was an experimental study using a posttest-only control group design. Mangrove bark was extracted by using the maceration method. Cigarette smoke exposure was performed using an electric cigarette. Liver inflammation is assessed histopathologically.

Results: Significant results ($p < 0.05$) were found between the control and cigarette groups as well as the cigarette and bark extract + control groups. The results were not significant ($p > 0.05$) between the control and the bark extract + cigarette.

Conclusion : The administration of mangrove bark extract has a potential to protect the damage of pancreatic male white rats (Rattus norvegicus) Sprague Dawley strains exposed to cigarette smoke.

Keywords : Antioxidant, Anti inflammation, Mangrove, Smoke cigarette

INTRODUCTION

One of the basic human rights that must be met is getting free access to good quality of air. Human needs about 10-20 m^3 air per day. However, in some places the need for air cannot be met because of polluted air due to increased cigarette consumption. Cigarette is one of the air pollutants causing inadequate human air requirements.[1]

Cigarette smoke causes pathological conditions such as inflammation, proteolysis, and oxidative stress. Oxidative stress is a process of shifting the balance of oxidants and antioxidants that tend to shift to oxidants. Oxidative stress is caused by reactive oxygen species (ROS). ROS is an oxygen molecule produced from normal cell metabolism. In low to moderate concentrations, ROS is useful for cell physiology process. However, in high concentration, it can be detrimental, so that in the physiological state of the oxidative stress, it should be prevented.[2,3]

Previous studies had shown that Rhizophora apiculata contain different active compounds. The results from Gas chromatography-mass spectrometry (GC-MS) analyses indicated that mangroves have a number of natural antioxidants such as phenolics, alkaloids, glycosides, essential oils, and other organic compounds.[4]

MATERIAL AND METHODS

This research was an experimental study using a posttest-only control group design. Data was taken at the end of the study after treatment, the groups were considered similarly before being treated. At the end of the study, the results in all three groups were compare

The independent variables in this study were bark stem extract given to white rats (Rattus novergicus) male Sprague Dawley strain and exposure to cigarette smoke. The dependent variable in this study was a picture of pancreatic histopathology exposed to cigarette smoke. The intermediate variables that can be controlled were the types of mice, the age of mice, food mice, beverage mice, and dosage of mangrove bark extract. An uncontrollable intermediate variable is the absorption of mangrove stem extract in mice and rats response to cigarette smoke exposure.[5]

The sample in this study was male white rat pancreas (Rattus novergicus) Sprague Dawley strain. This study used three treatment groups and each group consisted of 10 rats based on Federer's formula and drop out. Group one is a group that is given only regular food and drinks just like 2 other groups. Group two were rats given 2 cigarettes for 1 hour per day for 30 days. Group three were mice were given mangrove stem extract at dose 56,55 mg/kgBB and given 2 cigarettes for 1 hour per day for 30 days.[6]

Mangrove bark was extracted by using the maceration method. The bark was obtained from East Lampung shoreline. The part of the plant was separated between the stem, bark, and root. The bark of mangrove stems was washed and then dried in open air. As much as 600 grams of mangrove stem skin extract was washed and cut into pieces. The skin of mangrove stem was milled using grinding machine until it became powderous. Oil grazed Simplicia bark powder was immersed in a 95% ethanol solvent of 1.5 L for the first 6 hours and stirred occasionally, then continued for 18 hours. The mixture with 95% ethanol solvent was filtered with filter paper to obtain the

filtrate. The obtained filtrate was evaporated by rotatory evaporator 50°C. One ml of mangrove stem bark extracts were taken and then allowed to dry for 24 hours at room temperature. The dried yields were weighed to obtain the specific gravity and volume of 0.0872 g/ml and 52 ml, respectively. The dose of mangrove root bark extract used was 56.55 mg/kgBW. Thus the mangrove stem extract given to mice weighing 200 g was 11.31 mg.[7]

Cigarette smoke exposure was performed using an electric cigarette. The animal was terminated by cervical dislocation method by using chloroform anesthesia. Histopathologic examination of the pancreas was observed with 400x magnification in five fields of view. The interpretation was applied with score 0 (zero) if no inflammation of cell was found and score 1 if inflammation of cell was found.[8,9]

Statistical data analyses were performed using the Statistical Package for the Social Sciences (SPSS) program with bivariate analysis type. The statistical test used in this study was a chi-square test because this research has unpaired categorical comparative analysis. If the requirement of chi-square test is not met, then fisher test is used as alternative statistic test.

RESULTS

Three groups of histopathologic images were shown in Figures 1, 2 and 3. In the first group, the pancreatic parenchyma cells of rats in normal circumstances were shown by the pancreatic parenchymal cell arrestment with no massive necrosis cell, atrophy, edema, fibrosis, or inflammatory cell. In the second group, cells with edema and atrophy and massive inflammatory cells were found. However, no cells were found to be necrotic. In the third group, cells that experienced edema and atrophy were still observed, but no necrosis cells were observed. In this group, fewer inflammatory cells were observed compared with the number of inflammatory cells in group two.

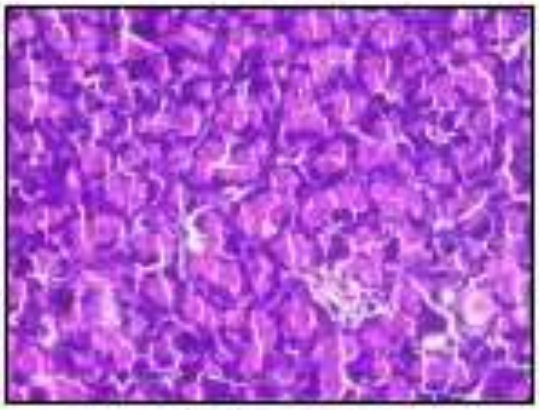

Figure 1. Group one

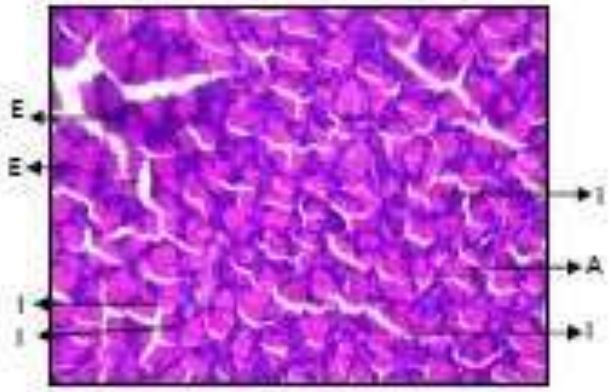

Figure 2. Group two (E: Edema, A: Atrophy, I: inflammatory cell)

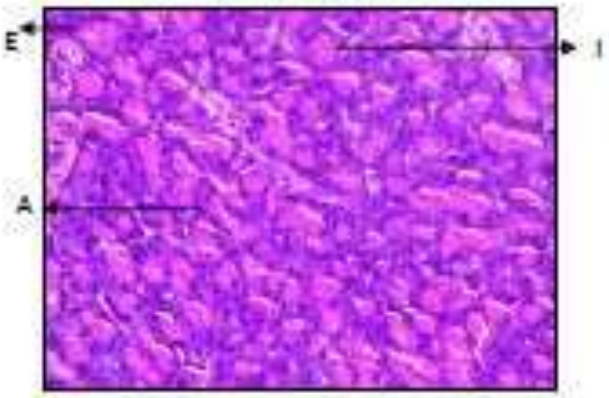

Figure 3. Group three (E: Edema, A: Atrophy, I: inflammatory cell)

By employing univariate analysis, an average number of pancreatic inflammatory cells with 400x magnification in five fields of view were obtained. The number for group one, group two and group three were 0.40 ± 0.52, 3.60 ± 0.69 and 2.10 ± 0.74, respectively (Table 1).

Table 1. Histopathological Analysis of Rat Pancreas (Magnification 400x in five field of view)

Group	Mean ± SD
Group 1 (control)	0,40 ± 0,52
Group 2 (cigarette)	3,60 ± 0,69
Kelompok 3 (ciagarette +mangrove)	2,10 ± 0,74

An average number of pancreas inflammatory cells with 400x magnification in five fields of view were obtained by applying bivariate analysis. There were 3 cells or 50% with an expected value of less than 0.05. After applying the chi-square test, the requirements for the 2x2 table were not met so that fisher test was performed. The results were shown in tables 2, 3, and 4.

Table 2. Chi Square Test G1 and G2

	No inflammatory cell		Inflammatory cells		P Value
	n	%	n	%	
G1	10	100	0	0	0,001
G2	0	0	10	100	
Total	10	50	10	50	

Table 3. Chi Square Test G1 and G3

	No inflammatory cell		Inflammatory cells		P Value
	n	%	n	%	
G1	10	100	0	0	0,105
G3	7	70	3	30	
Total	17	85	3	15	

Table 4. Chi Square Test G2 and G3

	No inflammatory cell		Inflammatory cells		P Value
	n	%	n	%	
G2	0	0	10	100	0,002
G3	7	70	3	30	
Total	17	85	3	15	

The results showed significant differences between group one and two ($p < 0,05$), as well as groups two and three ($p < 0.05$). However, statistically, there was no significant difference between group one and three ($p > 0,05$).

DISCUSSION

Microscopic description showed that showed normal pancreatic parenchyma cells indicated by tightly arranged cells and and the absence of necrotic cells, atrophy, edema, fibrosis, or massive inflammatory cells. Aquades was the material used in the treatment and without exposure to smoke cigarettes and mangrove leaf extract. Thus, aquades was not a material that acts as an aggressive factor in pancreatic cells.[6]

When group two was compared with group one, there was a significant difference statistically ($p < 0.05$). In group two, cells found to be edema and atrophy, but no cells were found to be necrotic. Massive inflammatory cells were also observed. This was consistent with previous studies that by employing a single clove cigarette for one hour in 30 days caused pancreatic cells damage in the form of atrophy, edema, but the absence of necrotic cells.[6]

The process of pancreatic histopathologic changes is caused by exposure to cigarette smoke compounds given to mice for 30 days. Exposure to

cigarette smoke contains two main compounds of nicotine and tar. Nicotine in cigarette smoke contains various types of free radicals such as nitric oxide and nitrite dioxide that convert oxygen into superoxide anions that will form hydrogen peroxide and subsequently hydroxyl radicals. The tar that acts as a smoke condensate and the total residue, enters the lungs as solid vapors. The type of free radicals contained from tar is a semiquinone that can reduce oxygen to superoxide anion. These free radicals result in the destruction of polyunsaturated fatty acids in the cell membranes and potentially lead to fragmentation of DNA that can be a risk for cancer cells formation.[10,11]

The process of inflammation is related to the presence of free radicals. The presence of free radicals can induce the formation of inflammatory radicals that can lead to further inflammation. An example is a nitric oxide, a radical produced by nitrite dioxide synthetase (iNOS) which acts as a second messenger in the inflammatory process. The formation of iNOS occurs as a response to inflammatory cytokines caused by free radicals. iNOS stimulates the formation of nitric oxide which results in further inflammatory processes.[12]

These results supported previous research that cigarettes can be an independent factor of pancreatic cell damage. In the past, cigarettes were known as cofactors of alcohol that resulted in the destruction of pancreatic cells. But after a retrospective cohort study, it was found that there was an average shift in the age of acute pancreatitis diagnosis. The diagnosis of pancreatitis in smokers stands five years earlier than nonsmokers. In another study with measurement of cigarette consumption with pack years, ie the number of cigarettes per day multiplied by 20 years (20 pieces/pack). Smokers with pack years <12 had a relative risk (odds ratio) of 1.35. While in pack years 12-35 and > 35 each have a relative risk of 2.15 and 4.59.[11]

Based on microscopic observation, there was no significant different ($p>0,05$) when group three compared to group one. This result was consistent with previous studies using mangrove bark extract as an antioxidant in naphthalene induced hepatic mitochondria.[13]

In a study conducted using mangrove bark extract as an antioxidant in naphthaline-induced hepatic mitochondria showed DPPH activity of more than 50% at a dose of 56.55 mg/kgBW. Previous research also showed that antioxidant activity using DPPH and ABTS methods was the most optimal on the skin of mangrove stem. Antioxidant activity (IC 50) of mangrove stem skin extract measured by DPPH and ABTS methods were 3,31 µgmL-1 and 18,47 µgmL-1, respectively.[13,14]

The anti-inflammatory activity by tannins is related to the nature of the active compound as a secondary antioxidant that captures free radicals. The gallate structure, polygaloil glucose (PGG), has five groups of galoil ester groups that inhibit the expression of iNOS activity. PGG also acts as an anti-inflammatory by inhibiting the formation of prostaglandin E2 (PGE2). PGE2 is inhibited by PGG formation by the inhibition mechanism of cyclooxygenase 2 (COX-2), an enzyme that will catalyze the process from arachidonic acid to PGE2.[12,15]

CONCLUSION

This study concludes that the administration of mangrove stem extract (Rhizophora apiculata) ethanol 95% with dose 56,55 mg/kgBB potency to protect damage of pancreatic cells of white rat (*Rattus norvegicus*) Sprague Dawley strain induced exposure to cigarette smoke.

The future direction of this study is to test further toxic dose of mangrove stem extract (*Rhizophora apiculata*) against protection from parenchymal cell damage of pancreas. Another suggestion is to compare the effects of mangrove stem extract (Rhizophora apiculata) on mangrove stem extract with different species. Examination of antioxidant activity such as superoxide dismutase (SOD) and malondialdehyde (MDA) as well as anti-inflammatory activity such as tumor necrosis factor-α (TNF-α) are also important to explore.

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