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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 *Avicenna 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.

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