Kholis 9

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Chapter 12
Drug Discovery: A Biodiversity Perspective

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12.1 Introduction

12.1.1 Current Health Issues

The complexity of health issues does not significantly reduce by the fast advancement of science and technology and the improvement of human civilization. As a matter of fact, there are more and more diseases, generative or degenerative, infectious or non infectious diseases affecting human's lives. Different factors have contributed to the current health issues which include but not limited to the increasing of human population and transmission, life styles, quality of foods, water and environment and the emerging and re-emerging microbial pathogens.

On the other hand, mankind keeps striving to find drugs not only for fighting the existing diseases but also current emerging ones. Furthermore, antibiotics or drugs that have been used to fight many diseases, constantly loose their effectiveness due to one reason or another. Human are always at a continuous endless battle between finding cure and diseases. Unfortunately, it is so obvious to deny that the speed of drug discovery is at a slower pace than the emerging of diseases. WHO reported that at least 30 new diseases emerged in 20 years period of time (World Health Report 1996). Therefore, it has become a necessity to search for alternatives to find different kinds of drug sources.

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12.1.2 Conceptual Framework of Drug Discovery from Biodiversity

The concepts of antigen versus antibody, venom versus antidote somehow taught us that Mother Nature provided us the cures for every disease. It is just a matter of how to find the right drug for particular disease which is already available in the nature. By saying so, it is our duty to explore nature, in a responsible manner, to discover drugs for various diseases both existing and ones might emerge in the future. This work should be done collaboratively among individuals with different expertises even among countries that would contribute according their capacities. Some contribute in terms of facilities or expertise, while some others contribute in the form of natural resources. Some countries are simply blessed with very rich biodiversity either on the land (terrestrial) or in the ocean or even both. The most important thing to keep in mind is that all parties should work together for the sake of the betterment of mankind. So that none would think that they are neither more superior nor more important than others.

This chapter briefly described the power of Mother Nature as the abundant sources to find drugs for different kinds of illnesses include the challenges associated with the drug discovery process. Different findings reported by researchers had shown us that most if not all organisms in our planet from simplest form of lives to the most complex ones, from microbes to plants, either on the land or in the ocean possess various types of active compounds that potentially can be used as medicines. We, human being just need to search and discover these virtues provided by God the Almighty and to use them all for the sake of mankind's prosperity in the best possible manners.

Drug discovery through screening process utilizing natural products can become a solution of the slow and expensive drug discovery process using conventional way (Hassig et al. 2014; Li and Vederas 2009; Harvey 2008). In the United States, approximately 50% of drugs approved by Food and Drug Administration (FDA) originated from natural products or their derivatives (Newman and Cragg 2012). The key point of this drug discovery method is that the method requires the availability of a large number of extracts and or compounds for screening purposes. This can be possibly made by exploring the richness of biodiversity. Mother Nature has been known as long as human history as very rich sources for various types of human needs including as medicinal sources.

12.2 Drug Discovery from Natural Products

12.2.1 The History of Natural Products as Medicinal Sources

Since centuries ago, human had been relying on natural products, plants in particular, to fulfill their basic needs for food, cloth and housing. Plants can also be used as

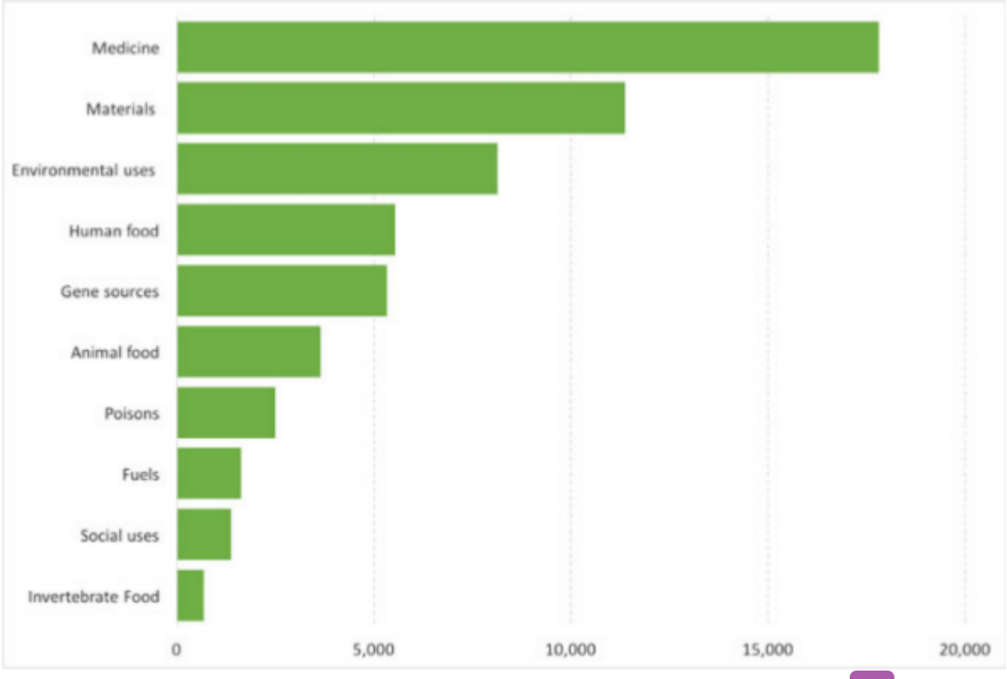

Fig. 12.1 Top documented uses of plant species. Graphic: Mongabay; Source: Royal Botanic Gardens Kew: State of the World's Plants 2017

poison on arrow for hunting, halusinogen in ritual, stimulant and medicines (Mann 2000). Plants had become very strong origin in traditional medicine practices in China, India and many other countries including Indonesia (Mittermeier et al. 2005). Long time before bioactive compounds were recognized pharmacologically, plants had been prescribed based on "similarity doctrine". For example, red color herbs were used to cure diseases related with blood and leaves with liver shape used to cure liver diseases (Sneader 2005). Traditional medicine practices generally use raw or processed materials according to traditions from generation to generation, such as well known boiled tea leaves or other herb formulas.

12.2.2 The Potential of Biodiversity as Medicinal Sources

There are about 391,000 species of vascular plants currently known to science, of which about 369,000 species (or 94%) are flowering plants, according to a report by the Royal Botanic Gardens, Kew, in the United Kingdom (Royal Botanical Garden Report 2017). Out of this number, only about 31,000 species have at least one documented use. These include uses for food, medicine, recreation, genes, poisons, animal food, and building material (Fig. 12.1) (Royal Botanical Garden Report 2017). At least 28,187 plants species are currently recorded as being of medicinal use (State of the World's Plants 2017).

12.2.3 Early Process of Drug Discovery from Natural Products

In later development, herbal medicines had been used in the form of pure bioactive compounds (Salim et al. 2008). Bioactive compounds found in plants are known as secondary metabolites. Secondary metabolites are differentiated into several classes which are alkaloids, terpenoids and phenolics. The discovery of plants secondary metabolites used wider later as medicinal materials either in the form of native compounds or modified ones (Samuelsson 2004). Isolation of bioactive compounds eventually led to discovery of various drugs such as cocaine, codeine, digitoxin and quinine (Newman et al. 2000; Butler 2004; Samuelsson 2004).

Drug discovery processes from plants are involving different area of research and analytical methods. Such processes were initiated by botanist, ethnobotanist, ethnopharmacologist and plant ecologist who collected and identified particular plants. The collected plants were the plants that had been known as medicines or had not been known at all. Phytochemists then do the extraction and do screening by appropriate pharmacological tests and start isolation and characterization of bioactive compounds. Drug discovery processes were then developed to molecular level through determination and implementation of molecular target screening tests which were physiologically appropriate (Balunas and Kinghorn 2005). The combination of different scientific fields mentioned above had become different interdiscipline science which was named as pharmacognosy. During its development, pharmacognosy includes a larger natural product research, bioactive compounds from various sources including bacteria, fungi and marine organisms.

12.2.4 Significant Values of Medicinal Plants in Drug Discovery Process

In last decade, pharmaceutical industries started reducing their drug discovery program from natural products due to vast variety of chemical structure and stereocenter of natural products, so their synthetic form were not economical (Koehn and Carter 2005). Pharmaceutical industries are more interested in modelled molecules, combinatorial and other synthetic chemistry that can produce millions of compounds (Ganesan 2004; Tan 2004). However, utilization of plants or other natural products are still significant parts in drug discovery. Bioactive compounds from natural products contain so many proptotypes of new bioactive compounds which had been proven to be more relevant in relation with new drug discovery process (Koehn and Carter 2005). Drug materials from plants are not only used as new drug materials but also as drug candidates with elevated activity (Kramer and Cohen 2004).

Nowadays, most of pharmaceutical industries' roles related to natural products are taken over by small biotechnology industries which focus on identification of

drug candidate compounds from natural product extracts and develop them to become drugs. Currently, there are many drug candidate compounds originated from plants are under clinical trial phases and introduced by the biotechnology industries, which some of them had become drugs that are commercially available. From the year 1981 to 2002, there were approximately 28% of new chemical compounds isolated from natural products or their derivatives. As many as 20% of them were chemical compounds analogues from natural products. Other researchers reported that in the United States, approximately 50% of drugs approved by Food and Drug Administration (FDA) originated from natural products or their derivatives (Newman and Cragg 2012). Natural products were also as initial point of synthesis of new synthetic compounds (Newman et al. 2003).

The nineteenth century was the beginning of the isolation of a number of other alkaloid compounds from medicinal plants, such as atropine (*Atropa belladonna*), caffeine (*Coffea arabica*), cocaine (*Erythroxylum coca*), efedrine (*Ephedra* sp.), morfine and codein (*Papaver somniferum*), pilocarpine (*Pilocarpus jaborandi Holmes*), physostigmine (*Physostigma venenosum*), quinine (*Cinchona cordifolia mutis* ex Humb), salicine (*Salix* sp.), theobromine (*Theobroma cacao*), theofilline (*Camelia sinensis*), and tubocurarine (*Chondrodendron tomentosum* Ruiz & Pav.). Up to now, isolation and characterization of new bioactive compounds from plants are still ongoing (Sneader 2005).

Artitir is a potential antimalarial compound and is as artemisinin derivative, sequiterpene lactone isolated from Artemisia annua L. (Asteraceae) (Graul 2001). Galantamine, drug for Alzheimer, discovered from lead ethnobotany and isolated for the first time from Galanthus woronowii Losinsk (Amaryllidaceae) in Russia in the year 1950 (Pirttila et al. 2004). Nitisinon was new drug from plant that works for rare generative disease, tyrosinemia, showing positive effect in lead structure. Nitisinon was a modification of mesotrione, a compound from Callistemon citrinus Stapf. (Myrtaceae) (Frank and Smith 2003). Tiotropium was recently released in US market for curing chronic obstructive pulmonary disease (COPD). Tiotropium is an inhaled anticholinergic bronchodilator medicine, an atropine derivative isolated from Atropa Belladonna L. (Solanaceae) (Frantz 2005). Morphin-6-glucuronid is a morphine metabolite from Papaver somniferum L. (Papaveraceae) and was going to be used as morphine substitute due to much smaller side effect (Lotsch and Geisslinger 2001). Vinflunine was modified from Vinblastine from Catharantus roseus (L.) G. Don (Apocynaceae) as anticancer (Okouneva et al. 2003). Exatecan was camptothecin analogue from Camptotheca acuminata Decne. (Nyssaceae) and was being developed as anticancer (Cragg and Newman 2004). Calanolid was dipyranokumarine natural product isolated from Calophyllum lanigerum var. austrocoriaceum (Whitmore) p. F. Stevens (Clusiaceae), Malaysian tropical rain forest tree. Calanolid is an anti HIV drug with a unique work mechanism as reverse transcriptase non-nucleoside inhibitor of type 1 HIV and effective against HIV AZT-resistant strain (Yu et al. 2003).

In addition to direct use as medicinal materials, bioactive compounds from natural products can also be used as drug precursors, prototypes for modification of synthetic compounds or pharmacological markers. Out of 244 identified drug

prototypes, 56 prototypes (23%) of them were secondary metabolites originated from plants (Sneader 2005). Bioactive compounds as drug precursors can be altered into preferred compounds through chemical modification process or fermentation methods (Salim et al. 2008).

By employing advance organic chemistry techniques, pharmacist can make analogue structure of natural products to obtain safer and more potential drugs. New produced compounds sometimes possess new pharmacological properties that can be categorized as derivative compounds. Podofillotoksin, camptotesine and guanidine were some examples of prototypes that their analogue had exactly the same pharmacological properties, while atropine was a prototype that its analogue had different pharmacological properties (Salim et al. 2008).

Bioactive compounds as pharmacological markers help researchers to understand work mechanism of intracellular signal transduction and biological mechanism related with certain diseases, so that better drug design can be achieved. For examples: Genisteine, an isoflavone naturally found in soybean (Glycine max Merr.) is an inhibitor of various protein tyrosine kinase (PTK), an important enzyme in intracellular signal transduction (Grynkiewicz et al. 2000). Different 12,13-diesther forbol also possess capacity to act as tumor promotor or activator protein kinase C (PKC) (Kazanietz 2005).

12.2.5 Challenges in Drug Discovery from Plants

Despite of many success stories of drug discovery from plants, following efforts remain facing many challenges. Pharmacognosist, phytochemist and natural product researchers still have to increase quality and quantity of compounds that enter drug development to be able to compete with chemical drug discovery business. Drug discovery process sometimes need about more than 10 years and spend about more than USD 800 million (Dickson and Gagnon 2004). Many of drug candidates considered failed after spending so much time and money. As a matter of fact, only one in 5000 drug candidate compounds which were entering clinical trials and accepted as drug. The steps mentioned above include identification, optimization and development of drug candidate compounds and then enter clinical trials (Balunas and Kinghorn 2005).

Those facts showed that drug discovery from plants requires longer time and more expensive compared to other drug discovery methods. Thus, many of pharmaceutical industries reduce their natural product research activities (Koehn and Carter 2005). To overcome such issues, it is important to develop a faster, better technology and or strategy, especially in increasing number of plant collection, screening methods, isolation of compounds and development of natural products and their derivatives (Do and Bernard 2004). Other challenge was limited availability of natural product extract, so it is not sufficient for optimization and development of drug candidate compounds. The challenge is also faced at clinical trials phases, so that natural products should be combined with synthetic compounds or chemicals.

Other technique is by developing extract library from natural products that combines natural products features with combinatorial chemistry (Butler 2004). Drug discovery through screening process utilizing natural products can become a solution for the slow and expensive drug discovery process using conventional way (Hassig et al. 2014; L[illegible]nd Vederas 2009; Harvey 2008). The key point of this drug discovery method is that the method requires the availability of a large number of extracts and or compounds for screening purposes. This can be possibly made by exploring the richness of biodiversity. Mother Nature has been known as long as human history as very rich sources for various types of human needs including medicinal sources.

12.3 Extract Library

Extract library is a collection of extracts containing active compounds from natural products used for screening process of target biology. Although this looks simple, however development of extract library requires good understanding regarding modern paradigm of drug discovery process (Quinn 2012). High quality extract library leads the way in discovery of natural products that can be developed to become drug and early identification point for chemical compound optimization. Current drug discovery process is supported by HTS ability for larger chemical library (up to one million compounds), so that shorten the cycle of drug discovery project. Screening can be performed in 384 or 1536 formats of sample wells with volume of each sample approximately 2–20 μL in each well. Target-based screening and special technology platform is very suitable with current HTS. Unfortunately, this method applies only for pure synthetic compound library and not for crude extracts that contain hundreds of compounds (Quinn 2012).

The concept of extract library was firstly introduced when the High-Throughput Screening (HTS) concept was firstly recognized by pharmaceutical industries (Pereira and Williams 2007). Crude extract library has several benefits, such as cheap and easy preparations, minimum preparation time and possess high diversity level. However, crude extract library has some limitations such as natural physical form is not suitable for automated solution system (too concentrated), minor metabolite cannot be detected, requires longer time and continuous sample supply for isolation and identification of active compounds, the isolated compounds might have been known or non-targeted chemical compounds (Liu 2008).

Natural products pure compounds library was developed to cover the shortage of crude extract library and could become solution for drug discovery research from natural products suitable for high-throughput method (Bindseil et al. 2001). Similar to small synthetic molecules library, pure compounds extract library was designed with strategy adjusted to representative limitation of the desired chemical range (Brenk et al. 2008). Infrastructure and more sophisticated chemical informatics also need to be developed to determine potential extracts containing the desired compounds and to eliminate the undesired compounds. Potential minor components can be detected and included in screening process depend upon final detection or

Table 12.1 Top ten countries with their respective biodiversity in numbers

Country	Birds	Amphibians	Mammals	Reptiles	Fish	Vascular plants
Brazil	1753	1022	648	807	4521	56,215
Colombia	1826	771	442	601	2053	51,220
China	1240	411	551	478	3330	32,200
Indonesia	1615	347	670	728	4682	29,375
Mexico	1081	377	523	916	2602	26,071
South Africa	755	128	297	447	2059	23,420
Venezuela	1364	360	363	405	1709	21,073
United States	844	300	440	530	3067	19,473
India	1180	390	412	689	2465	18,664
Ecuador	1588	539	372	444	1098	19,362

Source: Plant data is from the World Conservation Monitoring Centre of the United Nations Environment Programme (UNEP-WCMC 2004). Species Data. Fish: Fishbase; Birds: Birdlife International; Amphibians: AmphibiaWeb; Mammals: IUCN; Reptiles: the Reptile Database (Butler, 2016)

isolation method used (Roy et al. 2010). Although some pure natural product compounds are already commercially available in affordable price, however majority of compounds need to be isolated individually or obtained from researchers through establishment of consortium. However, the cost, time and related resources with isolation and characterization of single compounds can be reduced.

Final objective of extract library is to obtain resource of different compounds for HTS evaluation (Dandapani et al. 2012). Compounds diversity is strongly correlated with biodiversity. Keep in mind that 17 countries with megabiodiversity in the world (Australia, Brazil, China, Colombia, Democratic Republic of Congo, Ecuador, India, Indonesia, Madagascar, Malaysia, Mexico, Papua New Guinea, Peru, Philippine, South Africa, United States of America and Venezuela) possess more than 70% of the entire biodiversity. Currently, there are approximately 391,000 species of vascular plants currently known to science, of which about 369,000 species (or 94%) are flowering plants, according to a report by the Royal Botanic Gardens, Kew in the United Kingdom (Royal Botanical Garden Report 2017). Out of this number, only about 31,000 species have at least one documented use. These include uses for food, medicine, recreation, genes, poisons, animal feed, and building material (Fig. 12.1) (Royal Botanical Garden Report 2017). In other words, with this large collection of plants available in the world, millions of extracts can be generated. The full list of countries with richest biodiversity in the world is shown in Table 12.1 (Royal Botanical Garden Report 2017).

Indonesia in particular has abundant natural products potential as drug and cosmetics materials both on land and in ocean. According to a report, Indonesia was listed as the big four of the richest country in the world in biodiversity both in the ocean and on the land as shown in Table 12.1 (UNEP 2004). One of the very popular collections of Indonesian herbal is Herbal collection managed by the Main Center for Research and Development of Plant Medicine and Traditional Medicine under the Ministry of Health of Indonesia. This sanctuary is located in Tawangmangu, Karanganyar, Central Java. It is the home of about 2000 Indonesian

medicinal plants. Hundred of thousands of extracts can be generated from this single collection employing various solvents with different polarity.

The basic concept of this extract collection is that there is no single fraction discarded from every step of extraction processes. With the assumption that every extract has its own medicinal potential(s), either the one(s) generated from polar or non polar solvents. These collections can be utilized by different research institutions, universities or pharmaceutical industries for various research and development activities at different stages of research either basic, pre-clinical or clinical studies.

With the assumption, let say about 500 extracts or fractions can be collected from a single plant, there will be around one million extracts or fractions can be collected from 2000 medicinal plants available as in the sanctuary mentioned above (Audah 2015).

12.3.1 Sample Collection

Biota sample collection can be in the forms of bacterial culture, plant collection or marine biota. Some approaches in sample collection can be based upon their uses as traditional medicines or can be performed through random selection. Regardless of the basis of their collection, documentation, for examples place and time, and curation, for examples different parts of plants, are very important aspects for research continuation and downstream activities. Collection that is nationally programmed will ease the related research with conservation and understanding about the genetic resources. Species taxonomy identification is very important to increase the opportunity to discover new species that contain new compound(s) and to avoid the previously known one(s).

The latest biota sample collection strategy requires much less material compared to the requirement of the previous screening process. Development of screening technology, particularly the test with 384 and 1536 wells, approximately 200 mg extract is sufficient for screening several tests in HTS. With the development in spectroscopic structure elucidation, one milligram of compound is already sufficient, either for structure elucidation or biological profile for primary HTS test and some selectivity tests (Quinn 2012).

12.3.2 Extract Collection

Extracts can be collected from the whole biota or particular subsample according to the need for screening. Extraction from the whole biota ensures that all extracts are available for screening, isolation and chemical structure elucidation. However, degradation of compounds can occur to the extracts stored in a longer period of time. The need of time and solvent are larger compared to the extraction procedure of subsample of biota sample. Dried and ground plant sample (simplisia) can maintain

the compound integrity. Moisture should be controlled to prevent sample damage. High moisture increases the chance of fungi and microorganisms to grow. Sample should be stored in each special container and barcoded.

Biota sample can be processed to become several form of extracts suitable for screening such as (1) crude extract which is extraction using organic or mixture of organic solvents; (2) prefractination crude extract library, which is crude extract that is fractionated using Solid Phase Extraction (SPE) or conventional liquid chromatography technique or combination of both; and (3) pure compound of natural products (Quinn 2012). Furthermore, extract collection requires representative storage room at every extract collection center. Ideally, this type of facility should be located at government institutions, universities or companies appointed as business partners that have the ability to do such big task. Extract collection in smaller numbers can be done in each research center or laboratories. One thing that needs to keep in mind is that all the procedures should be kept uniform (standardized). All these extract collections, either the small ones or the large ones should be well recorded in a database so that the database can be easily managed or organized, utilized and monitored. The database should be accessible to different parties with the permission of the authorized body.

12.3.3 Fractionation and Dereplication Handling

When an extract shows biological activity, fractionation is required to separate groups of compounds which have physical and chemical properties similarity such as solubility and acidity (Hughes et al. 2011). Every fraction is tested and active sample should be repeatedly fractionated to increase purity of the compounds. Test and fractionation process should be continuously performed until pure compounds responsible for particular biological activity obtained. However, repeated fractionation process should be prevented by dereplication process (Katiyar et al. 2012). Dereplication refers to the process of screening active compounds early in the development process to recognize and eliminate those compounds that have been studied in the past, thereby proactively decreasing the number of structures that will need to be fully elucidated and minimizing the amount of time spent on testing (Gaudêncio and Pereira 2015).

The isolated active compound should be purified and its molecular structure should be elucidated. If the active compound was commercially potential, the synthesis of its analog compound and structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) studies were required (Guo 2017). However, if the natural source is commercially available to produce adequate active compound, so the synthesis that requires high cost production process and with the complexity of the stereochemistry of active compound can be avoided. For example, anticancer compound Vincristine, Etoposid and Taxol had already been synthesized, these compounds have chiral atom which is more economic when it is produced by extraction process from natural products compared to chemical

synthesis (Demain and Vaishnav 2011). Plant conservation should also be taken into consideration, for example, isolation of Taxol anticancer drug from Yew Pacific plant's bark can cause extinction of the plant. So, nowadays Taxol can be obtained through semisynthetic process from Yew plant's leaf from Europe and America (Juyal et al. 2014).

12.3.4 Recollection

Recollection process should consider the conservation concept of species and habitat of collected subjects, because target compound research progressively will require large amount of material, it will give impact to environment. Therefore, it is important that initial collection is well documented. Previous sampling process should be equipped with GPS data, complete documentation and good taxonomy knowledge (WHO 2005).

In conducting biodiscovery research, it should be considered that if recollection of sample in large amount can not be done, target compound should be able to be chemically synthesized. Continuous supply of drug material from plants in adequate amount is very important in fulfilling market demand. Utilization of plant cell culture can be an alternative method for compounds in which their synthetic process is not economical and only available from plant in small amount. Plants accumulate secondary metabolites at certain stages of development. By manipulating environment condition and growth media, desired secondary metabolites can be obtained instead of directly from the entire plant. For example, paclitaxel was successfully produced from fermentation technology of plant cell (Ochoa-Villarreal et al. 2016).

Database formation when the time of sample collection is very important to be adjusted with biodiversity conservation concept and also to trace sample through HTS. Database 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. This is also important to identify factors contributing to bioactivity, such as season, location and reproduction cycle stage (Atanasov et al. 2015).

12.3.5 Screening

In the past, drug discovery from plant bioactive compounds was a time consuming process. Even just for identifying structure of active compound, it took few weeks even years depend upon the complexity of the compound structure. Nowadays, the speeds of biological assay-based fractionations increase significantly due to the advancement of High Performance Liquid Chromatography (HPLC) tandemed with Mass Spectrometry (MS) or Liquid Chromatography-Mass Spectrometry

(LC-MS), Nuclear Magnetic Resonance (NMR) and High-Throughput Screening with robotic automation. Capillary NMR (cap-NMR) spectroscopy was a breakthrough in characterizing of rare compounds found in organisms (Schroeder dan Gronquist 2006).

Development of HTS technology accelerates extracts screening from plants. Nowadays, biological activity assay is not the limiting factor anymore in drug discovery process. As many as 100,000 samples can be tested in less than a week due to the advancement in data analysis system and the robotic automation usage (Butler 2005). However, plant extract library screening is still facing problem because of the compounds autofluorescence characteristic or the ability of UV light absorption that interferes reading results. To overcome this issue, pre-fractionation of extracts can be applied to reduce some of these problems. In general, HTS can also be equipped with computational screening method to identify and to avoid compounds that can possibly give false positive results (Walters and Namchuk 2003).

Researchers throughout the world are racing in finding new drugs for different illnesses, either the existing or the emerging ones. These include drugs for antibacterial and anticancer. Inappropriate use of antibiotics in some countries had caused antibiotic resistant to some strains, causing serious health problems. Nowadays, many antibiotics are not effective anymore against some infectious diseases caused by bacteria or any other microbes. Therefore, the effort in finding active compounds from natural products either in land or ocean becomes very important. The search also applied to find effective drugs for different types of cancer.

Various methods have been applied to evaluate or screen antibacterial activity in vitro from extracts or pure compounds. The most popular basic method is disk diffusion and broth dilution methods. Disk diffusion method has some advantages compared to other methods such as simplicity, relatively cheaper, and high reliability of the test results on some bacterial species and easiness of test results interpretation (measurement of zone of inhibition) (Balouiri et al. 2016).

In regards with cancer, research on anticancer from plants has become new attraction for researchers in the world. Many of natural products showed pharmacological potential that can be good starting point for discovery of anticancer drug. Vinblastin and Vincristin from plant *Catharantus roseus* has shown their effectiveness to cure cancer in human (Farnsworth and Soejarto 2009). Screening process for natural products with anticancer potential can be conducted using Brine Shrimp Lethality Test (BSLT). In the last 30 years, BSLT are commonly used for toxicity test of various natural products from plants (Mayorga et al. 2010).

BSLT is considered cheap and using less test materials. Since introduced for the first time, this in vivo test was proven to be representative as bioassay guidance for cytotoxic active fractionation and anticancer agent (Ahmed et al. 2010). In addition, other researches suggested that LC_{50} value obtained from BSLT correlately positive with the results of toxicity oral test in mice (Arslanyolu and Erdemgil 2006).

12.4 Indonesian Biodiversity in a Glimpse

Indonesia is a country that possesses more than 14,000 islands, located between Indian and Pacific Oceans. According to Fauna and Flora International (FFI), Indonesia is 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. This makes Indonesia become one of the richest country in the world in biodiversity.

One of potential plants as medicinal and cosmetics sources and widely spread along Indonesian coastline is Mangrove (Fig. 12.2) (UNEP-WCMC). Mangrove dan mangrove associates are very potential plants as medicinal sources (Bandaranayake 2002). Along roughly 90,000 km coastline, Indonesia is home of about 20 family with about hundreds species of mangroves and their associates. Indonesia has the largest mangrove forest or about 23% of total world mangrove forests (Giri et al. 2011). Figure 12.3 showed a mangrove conservation area in eastern coastline of Lampung, Indonesia which was previously an empty land. Today, this conservation area had attracted different species of birds and fishes that migrates from other places.

Since long time ago mangrove trees had been utilized either roots, branches, leaves, flowers and the fruits as food and medicinal sources. Qualitative phytochemistry analysis showed that leaf extract of Rhizophora stylosa and Avicenna marina contain flavonoid, terpenoid, alkaloid, flavonoid and glycosidic phenolic (Mouafi et al. 2014). Extracts of mangrove fruit also contain some bioactive compounds such as flavonoid, saponin and triterpenoid (Rohaeti et al. 2010).

Mangrove extracts had shown their activity against microbes or pathogen parasites in animals and plants (Batubara et al. 2010; Batubara and Mitsunaga 2013, Audah et al. 2018), including HIV (Rege and Chowdhary 2013) and Hepatitis-B virus (Yi et al. 2015). *Excoecaria agallocha* L can be used to reduce epilepsy,

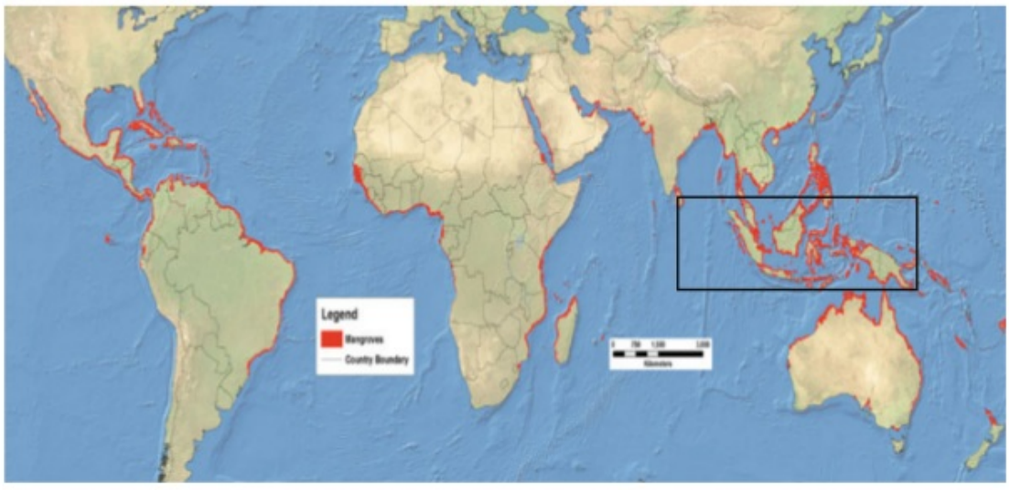

Fig. 12.2 Hot spots of world's mangrove. Inset is Indonesian mangrove hot spots (UNEP-WCMC)

Fig. 12.3 A mangrove conservation area in Eastern Coastline of Lampung, Indonesia (personal gallery)

conjunctivitis, dermatitis, leprosis, dermatitis, hematuria and toothache (Bandaranayake 2002). Mangrove extracts can also be used as antidiabetes (Gurudeeban et al. 2012), antinociceptive (Islam et al. 2012), antipyretic and antiinflammatory (Safari et al. 2016), anticancer (Singh and Kathiresan 2015), antiulcer (de-Faria et al. 2012).

Hibiscus tiliaceus can act as diuretic and laxative (Tambe and Bhambar 2016). According to Tanvira and Seenivasan (2014), some types of mangrove also can act as antivenom of snakebites. Mangrove extracts can also be used as mosquito's larvicide (Liem et al. 2013).

Up to now, mangrove research in Indonesia and worldwide is still very limited, especially for the purpose of drug discovery. This opens up opportunities for researches to start putting their efforts individually and collaboratively on mangrove research which also applies to mangrove's associates.

12.5 Concluding Remarks

Facing the challenges in discovering new drugs to fight vast diverse diseases, nature has given us abundant resources both in land and marine to search for. It's all up to us, scientists, researchers and all parties to work together to discover alternative for drugs to cure not only existing diseases but also ones that might emerge in the future. It's the time to complement and not to compete each other. People should share what they have for the betterment of mankind and make the world a better place to live for our generations in years to come.

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