I. Introduction

I.1. Significance of study

Since ancient times, nature has been an important source of medicines. This fact is illustrated by the large number of natural products currently used in medical practice, which were earlier identified through folklore knowledge of the medicinal properties of plants, animal extracts, and minerals (Amador *et al*., 2003). A recent analysis of natural products as sources of new drugs over the period 1981 – 2002 by Newman and collaborators (2003) indicates that even though 67% of the 877 small molecule new chemical entities (NCEs) are formally synthetic, but 16.4% correspond to synthetic molecules containing pharmacophores derived directly from natural products, whereas 12% can be categorized as natural product mimics (Cragg *et al*., 2006; Newman *et al*., 2003). Therefore, actually over 60% of the NCEs can be related to natural products in one way or another (Cragg *et al*., 2006).

Microorganisms are also defined as a prolific source of novel bioactive compounds from nature. They have yielded some of the very important pharmaceutical products such as the antibiotics penicillin and aminoglycosides, as well as the anticancer drugs anthracyclins and bleomycin. Marine compounds on the other hand are under-represented in current pharmacopoeias, but it is noteworthy to mention that the aquatic environment will become invaluable source of novel compounds in the future (Amador *et al*., 2003).

The ocean which covers almost 75% of the Earth's surface provides a huge biodiversity with potential as immeasurable source of natural products (Whitehead, 1999). But due to the lack of an analogous ethno-medical history, together with the

relative technical difficulties in collecting marine organisms, the development of marine-derived natural products as therapeutic agents is still in its infancy. Nevertheless, significant efforts to isolate and identify new marine-derived natural products have been made over the last few decades by collaboration of both pharmaceutical companies and academic institutions, accompanied by funding supports from government agencies (Amador *et al*., 2003). Systemic investigations directed towards the collection and characterizations of marine natural products, as well as the evaluation of their biological activity have been established (Christian *et al*., 1997; Amador *et al*., 2003). These efforts result in a large number of novel marine-derived compounds reported in the literature over the past decade (Mayer and Gustafson, 2003; Amador *et al*., 2003). Currently, some of these agents have entered preclinical and also clinical trials which can be expected that the number will increase in the future (Amador *et al*., 2003).

According to Proksch and co-workers (2002), the majority of marine natural products currently in clinical trial or under clinical evaluation are produced by invertebrates. The soft bodied, sessile or slow-moving marine invertebrates that usually lack of morphological defense structure like spines or a protective shell reflect the ecological importance of the chemical constituents to the respective invertebrates (Proksch *et al.*, 2002).

Many of these natural products also act as regulators of specific biological functions. Some of them have pharmacological activity due to their specific interactions with receptors and enzymes. They need to be highly potent on a molecular basis and retain a relatively low solubility since they become immediately diluted by large volumes of seawater (McConnell *et al*., 1994). It has been repeatedly shown that the accumulation of toxic or distasteful natural product is an effective

strategy to fight off potential predators (e.g. fishes) or to force back neighbours competing for space (Proksch *et al*., 2002; Proksch and Ebel, 1998; Proksch, 1999; McClintock and Baker, 2001). These secondary metabolites, which are produced as a result of evolutionary pressures to reserve or enhance an organisms ecological success (Proksch, 1999), have evolved into structurally diverse and usually stereo-chemically complex compounds with specific biological activity (Edrada *et al*., 2000) many of which belong to novel chemical groups not found in terrestrial sources (Carte, 1993).

Sponges in particular, have been reported as the prime source of marine bioactive metabolites (Blunt *et al*., 2005). Pharmaceutical interest in sponges was aroused in the early 1950s by the discovery of the unusual nucleosides spongothymidine and spongouridine in the marine sponge *Cryptotethia crypta* (Bergmann and Feeney, 1950, 1951). These nucleosides were the basis for the synthesis of Ara-C, the first marine-derived anticancer agent, and the antiviral drug Ara-A (Prokch *et al*., 2002).

Up to now more than 17,000 marine products have been described (MarinLit, 2006) of which sponges are responsible for more than 5300 different products (Faulkner 2000, 2001, 2002). The chemical diversity of sponge products is remarkable, in addition to the unusual nucleosides, bioactive terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides, and amino acid derivatives (which are frequently halogenated) (Sipkema *et al*., 2005).

The area of the Pacific Ocean comprising the Indonesian Archipelago, the Philippines, The Malaysian Peninsula, and New Guinea are considered to have the highest marine biodiversity in the world (Sheppard and Wells, 1988; Roberts *et al*., 2002). As the central and richest part of a larger Indo West Pacific region, Indonesia

alone has been reported to possess almost 830 sponge fauna species (obvious synonym not counted) (Van Soest, 1989) which exist in a high rate of dissimilarity among different area (Amir, 1992; Calcinai *et al*., 2005). Increased feeding pressure from fishes and possibly from predatory invertebrates such as mollusk, not to mention higher microbial infection in tropical areas might be the reason for more interesting and more diverse secondary metabolites found in sponges that live in this region (Proksch *et al*., 2002). Therefore, they might provide a huge source of potential new drugs from the sea or compounds to serve as lead structure for drug development. As new and more complicated diseases are encountered worldwide, the need for new chemotherapeutic agent is increased. On the other hand, Indonesian marine sponges as sources of bioactive natural products are still not yet well investigated. This fact could be observed as only few literature reports on bioactive marine metabolites from this tropical region have been published (Blunt *et al*., 2006). Therefore studies on bioactive substances from marine sponges collected from this area are highly required.

I.2. Literature background

I.2.1. Natural products of marine origin

The oceans, which cover almost 75% of the Earth's surface, contain a variety of species, many of which have no terrestrial counterparts. Thus represents an attractive source of novel bioactive natural products which prior to 1980, had been largely unexploited. Fortunately, technological advances over recent years have aided marine natural products chemists and also have fueled a rapidly growing interest in the hidden secrets of the oceans (Whitehead, 1999).

Bioactive marine natural products can be defined as biologically active products including primary and secondary metabolites derived from marine sources

(Gudbjarnason, 1999). Primary metabolites are essential to growth and life in all living systems, and are formed by a limited number of metabolic reactions. They serve as building blocks for synthesis of macromolecules, proteins, nucleic acids, carbohydrates and lipids. Secondary metabolites, on the other site are not essential to the life of the producing organisms. They are formed from primary metabolites, and many of them enhance the survival fitness of the organism. Some secondary metabolites may also serve as chemical weapons used against bacteria, fungi, insects, and large animals (Gudbjarnason, 1999).

Even though most of the “natural products” of interest to the pharmaceutical industry are secondary metabolites, the interest in products of primary metabolites such as various marine lipids, enzymes and complex heteropolysaccharides is increased (Gudbjarnason, 1999). In fact, the division between primary and secondary metabolites of sponges is a little hazy (Faulkner, 1984), as could be seen in an unusual carboxylic acid of phospholipids found in marine sponges *Higginsia tethyoides* (Ayanoglu *et al*., 1983; Faulkner, 1984), and amides of 2-methylene-β-alanine from *Spongia cf. zimocca* (Yunker and Scheuer, 1978; Faulkner, 1984).

Database provided by MarinLit (2002) shows that the source of new marine natural products is dominated by sponges (37%) followed by coelenterates (21%). Interestingly most of metabolites investigated in the preclinical and clinical trials are derived from sponges as well (Blunt *et al*., 2005) which somehow define their role as a potential source of new drug candidate.

Presumed biogenetic origin of the new substances from marine source has systematically assigned by Blunt and co-workers (2004) showing the domination of the terpenoid biogenesis pathway. This fact is not surprising regarding the chemistry

of the two largest groups examined; sponges and coelenterates are dominated by terpenoids as well (Blunt *et al*., 2004). Several studies show that these metabolites play an important role in antipredation, space competition, and control of epibiont overgrowth of the host organisms (Thakur and Müller, 2004).

In the early years of marine natural products research, there was less emphasis put on biological testing. But recently, focus on biological properties of these compounds is increased. Comparison of biological testing carried out on marine metabolites up to 2004 (Marinlit, 2004) describes the domination of anticancer activity (41%), followed by studies covering bioassay on mechanism of actions, structure activity relationship, etc. (21%) and antibiotics, including antifungal, antituberculosis and antimalarial (20%) (Blunt *et al*., 2006). This is somehow correlated to the fact that cancer is the major public health burden in the United States and in other developed countries. Currently, it has been reported that one of every four deaths in the United States is due to cancer (Jemal *et al*., 2004). A great unmet medical need in chemotherapeutic agents to overcome the development of multi-drug resistance has also lead the progress towards marine anticancer drugs. Increasing clinical importance of drug resistant bacterial pathogens such as in tuberculosis and MRSA (multiple resistant *S. aureus*) diseases has lead to the urgency for antibacterial research (Shu, 1998). Other progressing categories are in drugs for pain and asthmatic conditions where the interest is centered on *Conus* toxin and analogues of sponge sterols respectively (Newman and Cragg, 2004).

I.2.2. Sources, collection, screening and supply of marine bioactive metabolites

The process of discovering marine pharmaceuticals starts with the collection of marine organisms. This is often the most important step in the entire research

program because the quality of the collections influences all future research (Faulkner, 2000). The progress in scuba-diving techniques and deep-water collection instruments has been crucial, which has allowed the improvement of sample collection of previously inaccessible marine organisms (Amador *et al*., 2003).

In order to avoid adverse impact to the collection site, a combination of high biological diversity and density should be of consideration in finding the collection site. The collected samples must be accurately sorted, and similar organisms should rather be splitted than lumped together. Careful documentation is also very important for future re-collection. A sub-sample should be put aside for taxonomic studies, while the bulk sample must be rapidly frozen or stored in solvent to retard bacterial degradation of the specimens (Faulkner, 2000).

Crude extracts must be prepared for biological screening. Assays that require careful interpretation and provide a lot of information per assay are ideal, although they are more difficult to be used during a bioassay-guided fractionation. Considering the fact that some crude extracts may not be well tolerated by some bioassay, one may randomly isolate pure compounds on the basis of interesting chemical structures and screen libraries of pure compounds (Faulkner, 2000).

Despite that marine ecosystem is extremely rich and diverse; one should consider the limitation of resources in developing marine pharmaceuticals. In addition, the natural concentrations of many marine-derived pharmacologically active compounds are often minute and sometimes less than 10^{-6}% of the organism wet weight (Proksch *et al*., 2003). As an example, close to 1 metric tonne (wet weight) of the tunicate *E. turbinata* is needed to obtain approximately 1 g of the promising anticancer drug ecteinascidin 743 (ET 743) (Mendola, 2000; Proksch *et al*., 2003). Continued collection from wild source will soon lead to an overexploitation and