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# Red Sea Microbial Diversity for Antimicrobial and Anticancer Agents

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## Abstract

Marine natural products are valuable sources that could produce potential chemotherapeutic agents, and marine-derived organisms are widely recognized as important sources of these natural products with interesting biological activities. Furthermore, Red sea was recognized as a rich source of microbial diversity with unique metabolites that can be of pharmaceutical and medicinal importance. The discovery of novel active metabolites provides an intellectual challenge to examine new products for medicinal purposes. Although many bacterial strains were isolated from Red sea with potent bioactivities; however, the microbial diversity of marine environment such Red sea remained largely un-explored. Lately, it has been possible to easily collect marine samples, isolate, and further identify diverse marine organisms (e.g. bacteria, fungi, and algae and marine invertebrate) by modern molecular techniques. Based on a wide range of screening and extraction methods, a vast number of metabolites have been extracted from marine sources with potential biological activities such as antibacterial, anticancer, anti-inflammatory agents and many others. In this article, we have reviewed the previous studies that have been conducted to explore marine-derived bioactives of Red sea. In addition, we have illustrated the following aspects: (i) Red sea microbial diversity for antibacterial and anticancer agents; (ii) the conventional and molecular approach used for screening and characterization marine microorganisms; (iii) characterization of natural products of microbial origin, and (iv) drug development from novel marine-derived metabolites and clinical trials. Moreover, modern molecular technologies, meta-genomic, proteomic and bio-chemo-informatics approaches are adding more potency to the targeted search of bioactive compounds derived from marine microbes. The novel marine derived drug discovery is aimed to improve our understanding of natural resources, to improve public health awareness about the environmental microbial diversity and their importance for nutraceuticals, pharmaceutical, agrochemicals and food processing. Recently, the research area of microbial diversity of the Red sea and their metabolites has opened a new access for significant sources of more effective drug entities with low coast.

**Keywords:** Red sea; Microbial diversity; Metabolites; Antibacterial; Anticancer

## Introduction

Microbial infections are still the most common threat to public health, particularly in developing countries due to continuous increase of bacterial resistance to existing antibiotics and unavailability of an effective medicine. This has led to develop new antimicrobial agents to overcome this global crisis, and to assure the availability of an effective antibiotic at low cost. Marine-derived microorganisms particularly bacteria and fungi, are rich sources of natural products with interesting biological activities [1,2]. The pharmaceutical industry focused on the terrestrial environment for more than 50 years; however, marine habitats have remained nearly unexplored for their ability to extract medically important compounds. Almost 70% of earth's surface is occupied by marine environment, which offers unlimited potential for valuable biological and chemical diversity [3]. Furthermore, around 30,000 bioactive products have been isolated from marine-derived organisms and many of the extracted bio-products are currently either under pre-clinical or clinical trials for drug development [4,5]. In a most recent report, approximately 230 marine natural bio-products have been reported from 2009 to 2011, and most of them (about 102 compounds) have showed significant antimicrobial activities [6]. Interestingly, Khan et al. have reported about 272 marine bacteria, and 50% have showed an antagonistic activity at least against one of the four pathogenic strains: *E. coli, Listeria monocytogenes, S. aureus,* and *Vibrio cholerae* [7]. Marine discovery of active natural products is continuously increasing from day to day, which reached up to 1000 compounds per year [8]. Moreover, Red sea harbors almost 25 anoxic hot brine pools, where a massive number of thermophilic and halophilic bacterial and archaeal communities exist. These communities can live under extreme conditions such as high temperature, pressure, and salinity. The experimental results suggested that these bacterial and archaeal communities are rich sources of active enzymes and metabolites, which can be applied for pharmaceutical and biotechnology-based applications [9].

In previous studies, a number of bio-active compounds have been reported from marine sources with different degrees of action and potential, such as antibacterial, anticancer, antitumor, anti-proliferative, anti-microtubule, cytotoxic, photoprotective, and antifouling properties [10-12]. Marine-derived metabolites, like peptides (ribosomal and non-ribosomal), alkaloids, polyketides and terpenes, have demonstrated antimicrobial and antiviral activities [13,14]. Most of marine-derived microorganisms are characterized by unique physiochemical properties, which can help them to easily adapt to extreme habitats, and to tolerate the extreme conditions like high pH, temperature, pressure, oxygen level, light, nutrients limit, salinity and osmolality [15].

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Furthermore, Red sea is extremely oligotrophic water body [16], and it represents the worst-case of global warming scenarios in temperate oceans. Previously, the nutrient status or precipitation input of Red sea was described, but with no significant impact [17]. Mass deep-seawater turnover of Red sea is too slow [18], and Red sea was also known by a significant UV exposure [19]. Red sea was further characterized by an epipelagic zone, which has a high temperature (at the surface 24-35°C, and from 200 m to the bottom 22°C), and high salinity (approximately 41 psu) [20,21]. Moreover, Red sea was also characterized by unique coral reef systems, seasonal fluctuation of air, water temperature, and distinct level of available marine biota [22]. Therefore, these factors provided the opportunity to produce unique microbial diversity with unique primary and secondary metabolites. These marine natural metabolites differ from the terrestrial ones, and further can be exploited environmentally, biologically and pharmaceutically. Taken together, these studies and findings suggested that marine-derived microorganisms are valuable sources of by-products and enzymes, which can be of industrial, pharmaceutical, and environmental importance in the near future. One of the few studies was conducted to explore Red sea microbial diversity from Jordanian side of the Gulf of Aqaba [23]. In this review article, we have presented the microbial diversity of Red sea, antimicrobial/anticancer activity of microbial extracts, structures and function characterization of marine-derived metabolites. Summary of microbial diversity of Red sea, their metabolites, and their biological activities were depicted.

## Red Sea Microbial Diversity for Anticancer and Antimicrobial Agents

In the last two decades, cancer was the most common cause of increased mortality rate in both genders in the world. Many researchers have developed different anticancer drugs, and subsequently applied in clinical trials. However, drug side effects and anticancer resistance were the major obstacles for novel drug development. The mechanism of apoptotic cell death (Apoptosis) has been determined as a novel target for anticancer drugs [24]. Few studies have been conducted on marine microbial metabolites and its potential apoptotic effect on cancer cell lines. Kameyama et al. have reported Bisucaberin (Siderophore) as the first cytotoxic compound of the marine bacteria *Alteromonas haloplanktis,* which was isolated from 3000 m depth off the shore Aomori Prefecture, (Japan) [25]. Similarly, Skropet has described other compounds with significant cytotoxicity that were isolated from deep-sea *Bacillus, Streptomyces* sp., and *actinomycetes* sp. [26]. Cytotoxic effects against selected cancer cell lines have been determined in vitro with crude extract from marine-derived fungus *Rhizopus* sp. [27]. Recent studies have shown that many microbial extracts from the Red sea displayed significant cytotoxic and apoptotic activity. The deep-sea anoxic brines of the Red sea are one of the most extreme environments on Earth in comparison to overlying seawater. The anoxic brines are characterized by high salinity (increased from 4% up to 26%), high temperature (up to 70°C), increased concentration of heavy metals, and decrease in $O_2$ levels. Therefore, marine-derived microorganisms have the ability to tolerate and survive in these extreme conditions, since they are uniquely equipped with some self-defence systems. Red Sea microbial diversity (bacteria/fungi) are interesting objects for further studies to discover novel metabolites and new chemotherapeutic agents. Recently, Sagar et al. have shown significant cytotoxic activities of some halophilic bacterial species that were isolated from Red sea (Jeddah), and subsequently demonstrated the possible mechanisms of anticancer effect of bacterial extracts on different cancer cell lines [28,29]. While in another study, they have observed significant apoptotic and cytotoxic activities in cancer cell lines treated with marine bacterial extracts that were isolated from brine-seawater interface of the Red Sea. The experimental results clearly suggested that the bacterial extracts of *Halomonas* sp. and *Sulfitobacter* sp. strains showed significant anticancer activity. Wang et al. have reported that a new marine *Halomonas* sp. from the Red sea was able to produce cytotoxic compound hydroxyphenylpyrroledicarboxylic acids i.e. 3-(4-hydroxyphenyl)-4-phenylpyrrole-2,5-dicarboxylic acid (HPPD-1), and 3,4-bis(4-hydroxy-phenyl) pyrrole-2,5- dicarboxylic acid (HPPD-2) [30]. Similarly, Dolastatin A and Curasin A are antimicrotubule agents that were isolated from marine cyanobactera, and a number of their derivatives have shown a potent anticancer activity [31]. In another study, Fenical et al. have reported that marine *actinomycete* is a rich source of salinosporamide A [32], which is a potential irreversible inhibitor of 20S proteasome and can act as an anticancer agent. In general, a number of active molecules derived from marine organisms is currently under laboratory investigation and clinical trials for drug development [33].

On the other hand, antimicrobial medicines can be classified based on the microorganisms they act primarily against. For instance, antibacterial is used against bacteria, whereas antifungal is used against fungi. Marine algae or seaweeds are also valuable sources of marine natural products, and it has been studied as pharmaceutical agents [34]. A number of studies have been conducted on antibacterial and/or antifungal activities of marine-derived algal extracts against several pathogens [35]. Salem et al. have done screening studies for antibacterial activities on eight different seaweeds collected from the Red sea, Hurghada, Egypt [36]. The results have confirmed the susceptibility of Gram positive bacterial strains *B. cereus* and *S. aureus*, to the algal extracts was more than those of Gram negative strains such as *E. coli, E. fecalis, salmonella sp,* and *P. aeruginosa*. Furthermore, marine derived fungal endophytes refer to microorganisms that are able to colonize and live inside the internal tissues of marine organisms without affecting negatively on their host [37]. *Aspergillus versicolor* is a marine-derived fungi, was isolated from the inner tissue of green algae *Halimeda opuntia* of the Red Sea. Their ethyl acetate extract and isolated compounds were explored for antibacterial, anticancer, and antiviral (HCV) activities [38]. Therefore, microbial isolates extracted from marine-derived organisms and/or seawater of the Red sea have demonstrated promising antimicrobial and anticancer activities, and further need to be explored for novel drug entities for different infectious diseases.

## Red Sea Microbial Diversity for Active Metabolites

### Marine bacterial diversity

Marine microbial diversity mainly consists of archaea and eubacteria, which are important sources of novel metabolites [8]. Archaea group is extremophiles-bacteria that live under extreme conditions of marine environment. Extremophiles are a significant and cheap source for pharmaceutical, and producers of stable enzymes that can be used for industrial-based applications at high temperature and pressure [39]. On the other hand, marine bacteria include actinomycetes and bacilli, alpha-proteobacteria and gama-proteobacteria and many other anoxygenic anaerobes [40]. Marine-derived bacteria are a renewable source of novel metabolites with unique structures [41]. Marine bacteria occupy different niches in marine environment, which might has an influence on the chemical composition and nature of bacterial metabolites. Marine bacteria might be associated with inert or biotic surfaces, or planktonic (free-floating species), or they may inhabit the sediments of marine environment [42]. Abdulmohsen et al.

have isolated *actinomycetes* from different sponges samples that were collected from the offshore Fsar reef (Saudi Arabia) [43], these isolates demonstrated biological activities against bacteria, fungi and West Nile Virus protease. Furthermore, bacteria can inhabit the sediments of the ocean, and one most recent study has shown different bacterial isolates, namely *Brevibacterium* sp., *Moraxella* sp., *Cornyebacterium* sp., with antibacterial and/or antioxidant effect from the sediment of Gulf of Aqaba, Red sea [44]. In another study, they have explored the Red sea microbial diversity from Jordanian side of the Gulf of Aqaba [23]. More marine microbial isolates, their natural products, and their biological activities were depicted in Table 1 and microbial diversity without unexplored biological activities was showed in Table 2. However, in our study we are interested to focus on the microbial diversity of Red sea with antibacterial and/or anticancer activities. Only few studies have been done previously on the microbiology of the brine-pools of the Red sea that resulted in identification of only few microbial isolates [45-47]. However, phylogenetic studies have explored an unexpected high microbial diversity in the salty seawater edge of the Red sea [48,49].

## Red sea fungal diversity

Most of the marine fungi include *Ascomycota, Bacidomycota, Chytridiomycota, Deuteromycota,Oomycota, and Zygomycota* phyla [40]. Marine fungi harbor is also a potential source of secondary metabolites, and they often live as symbionts in algae or sponges. Most of the sponge associated fungi species belong to *Aspergillus* and *Penicillium* genera. Information on marine fungal strains is still little in comparison to terrestrial strains; thereby marine-derived fungi are also promising study objects for exploring bioactive natural compounds and novel drug discovery. Approximately 1.5 million fungal species existing in ecosystem [50], and only 5 to 10% has been formally described [51]. Fungi from marine environment assigned as either obligate or facultative. Facultative marine fungi are those that normally live in freshwater or terrestrial habitat, but they are also able to thrive in marine environment. Facultative marine fungi can be introduced into seawater by several natural ways, such as rain, wind, runoff soil, and few of them were able to adapt to live in marine environment and after long time became obligate marine fungi [52]. However, obligate marine fungi are those mandatory to cultivate and sporulate in a brine water/marine/estuarine habitat and permanently or periodically immersed in water [53,54]. Previous studies have recognized 444 species of facultative and obligate fungi, in which 360 species represent *Ascomycota*, whereas 10 species represent *Basidiomycota* and 74 species of mitosporic fungi [55]. Some marine fungi have been reported as rich sources of enzymes that are used in medicine and bioremediation [56,57], and new biosynthetic products have been extracted from marine-derived fungi [27]. Jaber et al. have investigated the existence of fungal life in brine seawater and sediments near northern end of Red Sea near Gulf of Aqaba (Jordan) [58]. The experimental results have identified five different isolates that belonging to three different genera, namely; *Aspergillus*, *Eupenicillum*, and *Penicillum* all of which are belonging to phylum *Ascomycota. Aspergillus* isolates have showed strong sequence homology of two *Aspergillus* sp. to *A. sydowii* and *A. wentii.* At the end, this study concluded that Gulf of Aqaba is a new geographical location for *Ascomycetes,* as facultative marine-derived fungi [58]. In another study, 26 species of *Ascomycetes* have been reported previously from the intertidal zone of Red Sea near upper

| Organism | Metabolite/ compound | Source | Activity | Reference |
|---|---|---|---|---|
| **Bacteria** | | | | |
| *Brevibacterium* sp., *Moraxella* sp., *Cornyebacterium* sp., | Crude extracts | Sediment of Gulf of Aqaba, Red sea | Antibacterial andantioxidant | [44] |
| *Vibrio* sp., | Aqabamycins A-G | From the surface of coral (*Sinularia polydactyla*) Red sea | Antibacterial and cytotoxic | [23] |
| *Halophilic* sp., *Chromohalobacter salexigens, Halomonas meridian, Idiomarina loihiensis, Chromohalobacter israelensis* | Crude extract | Brine pools of the Red sea and from sediment | Anticancer | [29] |
| *Halomonas Sulfitobacter* | Crude extract | Brine-seawater interface of the Red Sea | Anticancer | [28] |
| *Carteriospongia sp. and Dysidea sp.* | Scalarane sesterterpenes | Marine sponge Phyllo- spongia lamellosa collected from the Red Sea | Antibacterial and cytotoxic | [135] |
| *Gammaproteobacteria, Actinobacteria and Firmicutes* | Crude extracts | Soft coral Sarcophyton glaucum from the Red sea | Antibacterial and antifungal | [136] |
| *Nocardia* sp. | Chrysophanol 8-methyl ether, Asphodelin; 4,7'-bichrysophanol, Justicidin B, Ayamycin | Ras-Gharib coast of the Red Sea, Egypt | Antibacterial and antifungal | [137] |
| *Vibrio* sp. II | Aqabamycins A–G | Red Sea | Antibacterial and cytotoxic | [138] |
| ***Actinomycetes*** | Crude extracts | Sponges offshore Fsar reef Red sea | Antibacterial, antifungal and antiviral | [43] |
| ***Fungi*** | | | | |
| *Aspergillus versicolor* | Isorhodoptilometrin-1-methyl ether, Emodin, 1-methyl emodin, Evariquinone, 7-hydroxyemodin 6,8-methyl ether, Siderin, Arugosin C and Variculanol | Red Sea | Antibacterial, antifungal and Antiviral (HCV) | [38] |

**Table 1:** List of the Red sea microbial diversity, their natural products, and their biological activities.

| Organism | Source | Reference |
|---|---|---|
| **Bacteria** | | |
| *Methanosarcinales, Thermococcales,Methanococcales, Methanobacteriales, Halobacteriales, Nitrosopumilales, Methanobacteria, Methanococci, Methanomicrobia, Methanopyri, Victivallis vadensis, Salinibacter ruber, Pseudomonas filiscindens, Planctomyces maris, Ktedonobacter racemifer, Prolixibacter bellariivorans, Desulfobacteraceae bacterium, Francisella noatunensis, Robiginitalea biformata, Pseudomonas aeruginosa Pelagibaca bermudensis, Dokdonia donghaensis, Flexithrix dorotheae, Erythrobacter litoralis and Marinoscillum furvescens* | *Avicennia marina,* Gray mangroves of Red Sea | [139] |
| *Phaeocystidibacter marisrubri* | Red sea Sediments | [140] |
| *Nitrosopumilus maritimus* | Deep Red Sea | [141] |
| *Prochlorococcus sp.* | Red sea | [60] |
| *Methylocella*, *Methylocystis,Methylosinus, Methylocapsa Methylacidiphilum* | Red sea brine water interface | [142] |
| *Candidatus Synechococcus spongiarum,* *Cyanobacteria foliascens* | Red Sea sponge | [143] |
| *Cyanobacteria-Prochlorococcus* | Red sea water | [60] |
| *Poribacteria, Candidatus, Entotheonella, Spirochaeta, Nitrosococcus, Proteobacteria, Deferribacteres, Acidobacteria, Actinobacteria, Gemmatimonadetes, Bacteroidetes, Planctomycetes* | Red sea sponges | [144] |
| *Alphaproteobacteria and Bacteroidetes members* | Red Sea coral | [145] |
| *Prochlorococcus/Cyanobacteri, Alphaproteobacteria* | Red sea | 146] |
| *Alphaproteobacteria and Flavobacteria* | Red sea | [147] |
| *Endozoicomonas* | Red sea Coral | [148] |
| *Proteobacteria, Actinobacteria, Cyanobacteria, Deferribacteres, and Euryarchaeota* | Red Sea sediments | [149] |
| *Vibrio*,*Pseudoalteromonas*, *Serratia*, *Stenotrophomonas*, *Pseudomonas*, *Achromobacter,Chloracidobacterium* and *Endozoicomonas* | Red Sea coral | [148] |
| *Actinobacteria, Aquificae, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Dictyoglomi, Firmicutes, Deinococcus-Thermus, Planctomycetes, Thermomicrobia, Verrucomicrobia Acidobacteria, Chrysiogenetes, Deferribacteres, Fusobacteria, Gemmatimonadetes, Lentisphaerae, Nitrospira, Spirochetes, Tenericutes, Thermodesulfobacteria* | Red Sea Sponges | [149] |
| *Alphaproteobacteria, Gammaproteobacteria, Acidobacteria, and Actinobacteria,* | Red sea, Egyptian sponge | [150 ] |
| *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria and Epsilonproteobacteria, Actinobacteria, Cytophaga-Flavobacter/Flexibacter-Bacteroides, Firmicutes*, and *Planctomyces* | Red Sea Coral | [151] |
| *Halorhabdus tiamatea sp.* | Deep Red sea | [45] |
| *Haloplasma contractile* | Deep Red sea | [46] |
| *Desulfovibrio desulfuricans* | From Red Sea Coral | [152] |
| *Swampomyces armeniacus, Hypoxylon sp,* *Lineolata rhizophorae, Tethys kallichroma,* *Swampomyces egyptiacus* *Lulwothia grandispora.* | Red sea mangroves in Egypt | [153] |
| *Salinisphaera shabanensis sp.* | Red sea | [45] |
| *Halanaerobium sp.* | brine-seawater interface of the Kebrit Deep, Red Sea | [47] |
| *Alternaria alternata,* *Cladosporium cladosporioides* *Penicillium chrysogenum* | Red Sea Coast of Egypt | [154] |
| *Ascomycotina sp.,* *Deuteromycotma sp.* *Basidiomycete sp.* *Halosphaeria quadricornuta* *Leptosphaeria australiensis* *Periconia prolifica.* | South Safaga of Red Sea, Egypt | [59] |
| **Fungi** | | |
| *Aspergillus, Ustilago, Schizosaccharomyces, Phaeosphaeria, Chaetomium, Neosartorya, Nakaseomyces, Kluyveromyces, Postia, Saccharomyces, Filobasidiella, Kluyveromyces, Agaricus, Alternaria, Piromyces, Gibberella, Malassezia, Neurospora, Penicillium, Magnaporthe, Yarrowia, Debaryomyces* | Grey Mangroves from the Red Sea | [155] |
| *Sarocladium strictum* *Acremonium* sp | *Thuwal cold seeps in the Red Sea* | [156-160] |

**Table 2**: Red microbial diversity still not explored biological activities

Egypt [59]. Shibl et al. have also demonstrated that the Red sea harbors divers *Prochlorococcus* lineages by using molecular approach [60]. The genus *Prochlorococcus* is a widely distributed *picocyanobacterium* that represent high percentage of photosynthetic biomass and play a major role in primary production and carbon cycling.

## Screening microbes for active metabolites

There were a number of obstacles that limit the bioactive discovery progress particularly in the detection and characterization of marine microbial diversity and its bioactive compounds. The discovery progress was limited due to unstable cultivation techniques, optimum

parameter of growth and critical purification process. Recently, culture dependents and independent techniques, microbial metagenomics approach for characterization of microbes and metabolites, development of analytical tools and software for novel structure characterization, all of these helped to overcome many limitations.

## Marine microorganisms isolation

The standard procedure for the isolation of bioactive products of microbial origin includes several essential steps. The process starts with isolation of microorganisms from environmental samples like sediments, brine seawater samples, and seawater samples from the surfaces of eukaryotic organisms where microbial diversity thrived in the form of a biofilm. Previous studies have reported that some microorganisms are essential for eukaryotes survival and development [61,62]. Therefore, isolation of bacteria and fungi from surfaces of marine eukaryotes can significantly increase the chances of isolating active metabolites of bacterial origin. However, the researchers have faced some limitations of microbial bio-product development from marine epibiotic microorganisms. For instance, using bio-actives producing marine eukaryotes in large-scale production is very difficult because in some cases the eukaryotic organism should be killed for bioactive extraction process, and many of these organisms are non-cultivable in laboratory [63]. In contrast, the bioactive metabolites producing marine microorganisms are cultivable and can be easily manipulated in bioreactors [64]. In general, most of the bacterial strains were obtained previously by using the common streak plate method on nutrient agar plate [65]. Moreover, optimum cultivation conditions are mandatory for microbial growth and production of specific metabolites. Growth parameters depend on the temperature, pH, pressure, incubation time, media composition, and aeration. According to the standard method, most of the fungi can grow at 28°C in 1 to 3 weeks, and bacterial isolates can grow at 30°C for 1-3 days [66]. However, marine microbial isolates derived from Red sea can easily grow at high salinity and temperature because they have the ability to tolerate both high salinity (up to 25%) and high temperature (up to 70°C).

## Identification of microbial communities

Standard microscopic examination was the only way used previously to identify the number and type of bacteria in a sample. However, recent molecular techniques and sequence technologies have improved the identification and characterization of marine microbial diversity, and their primary and secondary metabolites [67]. For more than two decades, small ribosomal subunit s rRNA based gene sequence amplification approach has been utilized for microbial identification from environmental sample. Mainly 16S rRNA and 18S rRNA subunits have been utilized for bacterial and fungal characterization respectively [68]. The process of bacterial DNA extraction and PCR amplification was described with details in previous studies [69]. In addition, Zhang et al. gave detailed information of fungal DNA extraction and identification, and amplification of fungal ITS-rDNA Fragments [70]. Rastogi et al. have also assessed the physical properties of environmental microbial community by using molecular approach without microscopic examination [67]. Phylogenetic analysis has been used to identify the homology, likelihoods, and functional dynamic of the isolated organism with other existed microbial communities in the Red sea. Recently, 47 *actinomycetes* associated with marine sponges collected off Fsar Reef (Red sea, Saudi Arabia), were isolated and phylogentically identified based on 16 S rRNA gene sequencing, and were belonging to10 different actinomycetes genera [43]. Most recent study has developed the metagenomics for advanced characterization of microbial diversity from environmental sample. Behzad et al. have also suggested that only a broad and representative metagenmic database could help to identify a dynamic modal of the microbiota that reside in different niches of the Red Sea [9]. In addition, the metagenomic database also revealed the majority of non-cultivable environmental strains and other unknown microbial sp., which were unexplored due to unstable cultivation conditions and other non-advanced techniques. The enormous generated data also helped to identify a huge number of microbial genes, their mechanisms of adaptation, interaction within sp. and community, metabolite production and pathways as well as their pharmaceutical and biotechnology-based applications [9]. Therefore, direct metagenomic sequencing analysis of community DNA pools open the door for metatranscriptomics and metaproteomics analysis, and subsequently provided the related information to community physiology.

## Marine bacteria and fungi source for bioactive compounds

Red Sea marine environment is extremely intricate, and it contains an enormous diversity of life forms. Most of the ocean water sample contains approximately $\approx 10^6$ bacterial cells/ml [71]. Marine microbial diversity of bacteria and fungi are rich sources of novel bioactive compounds. Most of the marine bacteria and fungi can live in mutual association with soft-bodied organisms, which lack of self-defence mechanisms and lack skeleton in their structures. Therefore, the associated microorganisms begin to produce the secondary metabolites in the form of chemical defence mechanisms, which enable the host to survive/adapt in a stressful habitat [72]. Lately, the discovery of and the search for novel bioactive compounds from marine microbial diversity is of a great interest to drug discovery due to continues need for the development of novel and effective therapeutic agents. Blunt et al. [73] have reported an increased trend in the discovery of marine-derived microbial bio-actives and their secondary metabolites to be used for industrial and biotechnology purposes. In addition, It was reported during the period 2007 to 2009 most of novel marine-derived secondary metabolites of microbial origin that were isolated from different crude extracts of mangrove plants, coral, sponges and algal extracts; their biological activities (antibacterial or cytotoxic effect on cancer cell lines), and the mechanisms of their actions on the targeted cells.

## Marine secondary metabolites as an antimicrobial agent

The most important discovery of medicine was the antibiotic penicillin in 1928 [74]. Previous studies have shown that most of soil-derived microorganisms (bacteria or fungi) are valuable sources of unique bioactive metabolites [75]. As a result of emerging new pathogenic bacterial strains and development of bacterial resistant to existed antibiotics, there is a growing demand for development of new and potent antimicrobial agents against human infectious diseases. Currently, a number of governmental organizations provide their financial support and funding for similar research interests of marine-derived drug development. An Extensive work has been done on microbial isolation and screening from different geographical locations of marine environment on purpose of exploring novel bioactive metabolites. Following the isolation and purification of marine-derived metabolites, their biological activities were enhanced by methylation, esterification, and many others chemical methods. For instance, tauramide a lipopeptide was isolated from marine bacterium, *Brevibacillus laterosporus*. Subsequently, the antimicrobial activity of tauramide was enhanced by methylation and esterification. Therefore, tauramide activity was enhanced by peptide chain acylated at N-terminus [76,77]. Previous study has found that bacterial isolates that belong to *Marinispora* genera have the ability to produce the secondary

metabolites, lipoxazolidinone, with a significant antimicrobial activity [78]. Furthermore, hydrolysed Lipoxazolidinones becomes more effective against Gram-positive and Gram-negative bacteria. Lipoxazolidinones that based on compound 4-oxazolidinones also expressed greater antimicrobial activity [79]. Preliminary studies have shown that the bacterial strain, *Chromobacterial violaceum,* was also an important source for producing chromopyrrolic acid, which is a substrate for chlorinated bisindole pyrroles and lynamicins A-E synthesis [79]. Another important secondary metabolite, lycogarubins A-C, was also reported from myxomycete Lycogala epidendrum [80], and further the antimicrobial activity against Gram-positive and Gram-negative bacteria was identified. Isnansetyo et al. [81] have isolated a novel compound, zafrin (4b-methyl-5,6,7,8–tetrahydro-1 (4b-H)–phenanthrenone), from *Pseudomonas stutzeri* strain, which was isolated from Gulf of Karachi, Pakistan. The bactericidal effect of Zafrin was more efficient than ampicillin, vancomycin or tetracycline against *Bacillus subtilis*., and bacterial membrane was the target of its bactericidal due to its amphiphilic nature, disruption of bacteria membrane like nisin or triton X-100 antibacterial agents [82]. Similarly, Al-Gendy et al. [83] have also reported antibacterial compound, ayamycin, derived from secondary metabolites of *Nocardia* sp. ALAA 2000, which was isolated from the red alga (Laurenica spectabilis) in Ras-Gharib coast of the Red Sea, Egypt. Furthermore, the antimicrobial properties were also assessed against pathogenic Gram-positive, Gram-negative bacteria and fungi; the MIC value was observed between 0.31 to 1.57 mM. Hughes et al. [84] have identified antimicrobial compounds, marinopyrroles A and B, derived from secondary metabolites of *Nigrospora* sp. Those agents were very effective against methicillin-resistant S. aureus (MRSA), and also showed cytotoxic effect against human colon carcinoma cell lines. Marine fungi are also an important source of secondary metabolites, and it is also known to grow in mutualistic relationship with other marine organisms. A marine-derived fungus *Aspergillus* sp. was mainly grown on brown alga Sargassum horneri, which was collected from Gadeok Island, Busan, Korea. In preliminary study, dehydroychlorofusarielin B is a polyoxygenated decline derivative, which was extracted from marine derived fungus *Aspergillus* sp. This compound has shown an antibacterial activity against *S. aureus* and MRSA (methicillin-multidrug-resistant *Staphylococcus aureus*. Although many previous studies have reported a vast number of marine derived microbial diversity with potent biological activities, the marine environment of the Red sea is still largely unexplored.

## Marine microbial secondary metabolites for cytotoxic activity

Marine microorganisms are physiologically and taxonomically very unique from terrestrial ones, this distinction makes it more interesting and cheap objects for testing novel drug development. Cytotoxic drug development from natural product is one of the most interesting research areas that could contribute in improving human health. Initially, a novel bacterial *micromonospora* was isolated from soft coral of Indian Ocean, and was tested for the production of secondary metabolites from mycelial extract. Furthermore, secondary metabolite was characterized as a depsipeptide or thiocoraline, which is a DNA polymerase-a inhibitor [85,86]. In addition, secondary metabolites derived from *Curvularia* sp. have shown a potential cytotoxic effect on human tumour cell lines. *Curvularia* sp. was isolated from red alga Acanthophora spicifera, and its secondary metabolites have been identified as 14-membered phenyl acetic acid macrolactone, which also known as a macrolide apralactone A. Prachyawarakorn et al. [87] have reported some cytotoxic secondary metabolites derived from marine fungus *Pleosporales* srtain CRIF2. Moreover, secondary metabolites, cyclohexadepsipeptides a spicellamide A and spicellamide B, were identified from fungal strain *Spicellum roseum* that was isolated from sponge Ectyplasia perox derived from marine water of Caribbean island of Dominica. These peptides have shown cytotoxic effect against rat neuroblastoma B104 cell line, which was confirmed by CellTiter-Blue cell viability assay. Moreover, both compounds, spicellamide A and spicellamide B, have shown EC50 value 10.03 mM and 49.83 mM respectively [88] and more detail of anticancer agent obtained from marine environment mentioned in Table 3. Abdel-Wahab et al. [89] have reported two chemical compounds, spiromassaritone and massariphenonsecondary, derived from secondary metabolites of marine fungus *Massarina* sp. Both bioactive compounds have demonstrated antimicrobial activity against S. aureus, C. albicans, and control growth proliferation of human colon carcinoma cell line (HCT-116). In another study, marine derived fungal strain *Aspergillus carbonarius* was screened out from sediment samples of Weizhou island of China. The fungal isolates were cultured and grown in liquid broth media, and further two secondary metabolites were identified as carbonarones A and B. Furthermore, both compounds were effective against cancer cell lines of human leukaemia K562, murine leukaemia P388, human lung carcinoma A549, human promyelocytic leukaemia HL-60, and human hepatoma BEL-7402. Both compounds have shown EC50 values were 244.54 and 121.39mM against human leukaemia cell lines K562 among above all [90]. The cytotoxic secondary metabolite, aspergillusol A, was obtained from marine-derived fungus *Aspergillus aculeatus* CRI323-04A, which was isolated from marine sponge Xestospongia testudinaria near Phi Island, Thailand. Additionally, cytotoxicity against acute lymphoblastic leukaemia cell lines MOOLT-3 and human lung cholangiocarcinoma cell lines HuCCA-1 has been done with EC50 value 19 and 50 mM respectively [91]. A common terrestrial fungal strain Aspergillus versicolor was recovered from marine derived beach samples from Cottesloe, Western Australia. Fungal strain MST-MF495 of Aspergillus versicolor produced two novelcompound cotteslosins A and B further observed ctototoxicity against human melanoma cell lines (MM418c5), prostate cancer cell lines (DU145) and breast cancer cell lines (T47D) positively [92]. Similarly, endophytic fungus Aspergillus versicolor was isolated from red sea algae for production of Bioactive anthraquinones for antibacterial and antiviral activity against HCV [38] but stillanticancer activity unexplored, beside this red sea is habitat of number of fungi and bcaterial diversity but still less explored in point of view of anticancer agents.

## Conventional method for isolation of therapeutic agents from marine organisms

More than 60 year ago Bergman [93] has discovered the bioactive compounds from the marine environment. They isolate arabinose-based nucleosides as a naturally occurring nucleosides may be contains sugar in the form of ribose or deoxyribose. First marine derived bioactive product was Dedemnin B, it was passed several clinical trials on human disease. It was isolated from the marine drived *Trididemnum solidum*. Highly bioactive properties of compound in metabolites it may be reduced due to dilution factor of marine brine water. However, these bioactive compounds associated with other toxic compounds, during drug discovery its presence create major problems for researcher to understand the toxicity of compounds against cancer cells or normal cells. Most of the drug is more effective when increase dosed while drugs effective at reduced dosage it reflect the synergistic patterns of introduced compounds. This unique synergistic pattern of marine derived compounds might be offer new horizon of therapeutic agents and decrease the drug discovery rescue or inadequate safety issue. Number of microorganisms survive in mutual relationship with

| Red sea organisms | Anticancer agent | Cell line | IC50 (μM) | Reference |
|---|---|---|---|---|
| *cyanobacterium Moorea* | • Lyngbyatoxin A<br>• Aplysiatoxin<br>• Debromoaplysiatoxin | HeLa<br>HeLa<br>HeLa | 0.0092<br>13.03<br>3.03 | [161] |
| *Cyanobacterium Moorea producens* | • Apratoxin H<br>• Apratoxin A sulfoxide | NCI-H460<br>NCI-H460 | 0.0034<br>0.0899 | [162] |
| *cyanobacterium Leptolyngbya sp.* | • Grassypeptolides D<br>• Grassypeptolides E | HeLa<br>HeLa | 0.335<br>0.192 | [163] |
| *Sinularia polydactyla* | • 24-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate<br>• Durumolide C | HepG2<br>HCT<br>HepG2 | 6.1<br>8.2<br>1.0 | [164] |
| *Laurencia obtusa* | • Teuhetenone | MCF-7 | 22 | [165] |
| *Sarcophyton glaucum* | • Sarcophinediol<br>• Sarcotrocheliol acetate | HepG2<br>HepG2 | 18.8 ± 0.07 19.9 ± 0.02 | [166] |
| | • Deoxosarcophine<br>• Sarcotrocheliol acetate<br>• Sarcotrocheliol | MCF-7<br>MCF-7<br>MCF-7 | 9.9 ± 0.03<br>2.4 ± 0.04<br>3.2 ± 0.02 | |
| | • 6-oxogermacra-4(15),8,11-triene<br>• Sarcophinediol<br>• Deoxosarcophine | HCT116<br>HCT116<br>HCT116 | 29.4 ± 0.03,<br>19.4 ± 0.02<br>25.8 ± 0.03 | |
| *Sarcophyton glaucum* | • Sarcophytolol<br>• Sarcophytolide C<br>• Aromadendrene | HepG2<br>HepG2<br>HepG2 | 20 μM<br>20 μM<br>20 μM | [167] |
| | • Sarcophytolide B<br>• Sarcophytolide C | MCF-7<br>MCF-7 | 25 ± 0.0164 29 ± 0.030 | |
| | • 10(14)aromadendrene | PC-3 | 9.3 ± 0.164 | |
| *Sarcophyton auritum* | • Sarcophine<br>• 2-epi-sarcophine<br>• (+)-7a,8b dihydroxydeepoxysarcophine<br>• (1R,2E,4S,6E,8R,11R,12R)-2,6-cembradiene-4,8,11,12-tetrol | HepG2<br>HepG2<br>HepG2<br>HepG2 | 23 ± 0.12<br>20.6 ± 0.31<br>11 ± 0.22<br>21.1 ± 0.16 | [168] |
| | • Sarcophine<br>• 2-epi-sarcophine<br>• (+)-7a,8b-dihydroxydeepoxysarcophine<br>• (1R,2E,4S,6E,8R,11R,12R)-2,6-cembradiene-4,8,11,12-tetrol | MCF-7<br>MCF-7<br>MCF-7<br>MCF-7 | 22.4 ± 0.22<br>19.7 ± 0.24<br>18.4 ± 0.16<br>20 ± 0.12 | |
| *Petrosia sp.* | • (3_,7_,9_trihydroxycholest-5-en<br>• (3,7-dimethyl-2-(methylamino)-3H-purin-6(7H)-one<br>• (N-((3S,E)-1,3-dihydroxytetracos-4-en-2-yl)stearamide | MCF-7<br>MCF-7<br>MCF-7 | 153.0 ± 0.012<br>130.0 ± 0.017<br>23.0 ± 0.017 | [169] |
| | • (3_,7_,9_trihydroxycholest-5-en<br>• (3,7-dimethyl-2-(methylamino)-3H-purin-6(7H)-one<br>• (N-((3S,E)-1,3-dihydroxytetracos-4-en-2-yl)stearamide | HepG2<br>HepG2<br>HepG2 | 230.0 ± 0.031<br>145.0 ± 0.120<br>20.0 ± 0.120 | |
| *Sarcophyton glaucum* | • 7beta-Acetoxy-8alpha-hydroxydeepoxysarcophine | HepG2<br>HCT-116<br>HeLa | 3.6<br>2.3<br>6.7 | [170] |
| *Siphonochalina siphonella* | • Neviotine-C | PC-3<br>A549 | 7.9 ± 0.120<br>8.9 ± 0.010 | [171] |
| *Cnidarian Litophyton arboreum* | • Extract | U-937 | 6.5±2.3 | [172] |
| *Sarcophyton trochliophorum* | • Extract | HeLa | 5.2±1.2 | [172] |
| *Hyrtios erectus* | • Hyrtioerectine A | -- | moderately cytotoxic | [173] |
| *Palythoa tuberculosa* | • Palysterol G | MCF-7 | Induce apoptosis | [174] |
| *Siphonochalina siphonella* | • Sipholenone A | MCF-7<br>HepG-2 | 3.0<br>2.8 | *[175]* |
| *Callyspongia aff. implexa* | • Gelliusterol E<br>• Callimplexen A<br>• β-sitosterol | chlamydial inclusions | 2.3 | [176] |
| *Sargassum sp.* | • Fucoxanthin | MCF-7 | 11.5 | [177] |

| | | | | |
|---|---|---|---|---|
| *Spheciospongia vagabunda* | • (R)-20-hydroxy-N-[(2S,3S,4R)-1,3,4-tri-hydroxypentacosan-2-yl] octadecanamide<br>• (R,Z)-20-hydroxy-N-[(2S,3S,4R)-1,3,4-trihydroxytricosan-2-yl) non-adec-10-enamide | HepG2& MCF-7<br>HepG2& MCF-7 | 24.7& 6.8<br>21.3& 29.8I | [178] |

**Table 3:** Red sea organisms based anticancer agent and its $IC_{50}$ value against different cell lines.

other organisms like sponges, corals and marine plants. Sometimes these organism also important source of bioactive compounds because several microbes isolated from corals and sponges is similar source of compounds. In another reports most the marine invertebrates have not any physical defense system against surrounding predators in marine environment due this reason symbiotic association of microbes produce some bioactive compounds for defence purposes [94]. Symbiotic microorganisms of sponges and corals are archea and bacteria, actinomycetes, cyanobacteria, fungi and alage. Probable in some cases associated microorganisms have different bioactive compounds then host organisms [95]. Isolation and cultivation of the sponge symbionts and their nature of relationship have been reviewed from somewhere else [95]. In general marine environment is too diverse habitat with mangroves, deep sea sediments, coral reefs and hydrothermal vents, here microbes thrive in adverse condition and can be search microbes for beneficial bioactive metabolites. Number of marine microorganisms have been isolated from marine water, sediments and symbiotic host, and further cultivated in lab scale by conventional methods inherited from the soil microbiology [96].

## Uncultivable marine microbes detection

In general microbial species and community investigate by epi-fluorescence microscopy, scanning electron microscopy and molecular rRNA sequencing strategies to revealed the microorganism properties, It is culture dependent technique. Number of microorganism are not able to grow or culture at lab scale due to lack of optimised condition for unknown microbes. Its occurred due to cell damage by oxidative stress, inhibition by high substrate concentration, formation of viable cell depress the less culturable cells, iduction of lysogenic phages during starvation, and lack of cell-to- cell communication in artificial medium when grown in laboratory scale. In some cases to enhance the recovery of less culturable organisms by amendment of cell signaling molecules in growing medium [97,98]. One other method applied the concept of "extinction culturing" to isolate bacterial or fungal culture in small volume at low nutrient level than approximate laboratory media. Most of the marine bacterial strain are grown from this techniques like *Acidobacteria*, *Bacteroidetes, Proteobacteria, Planctomycetes* and *Verrucomicrobia* [98,99]. Uncultiable microbes might be grow in pure culture form if media amended with chemical components of their original natural environment. According above hypothesis, developed a natural marine environment in a specialized growth chamber designed for unculturable microbial growth. Marine sediment and water sample were serially diluted with autoclaved sea water, mixed with nutrient agar and placed in diffusion chamber and incubate in an aquarium to stimulate the environmental condition for optimum growth of uncultiable microbes, it much better recovery and growth rate than conventional methods [100].

Recently develop high throughput cultivation technique for detection and isolation of microbes, it based on combination of single cell encapsulation with microdroplet of gel. In this techniques individual cells encapsulated by small porous gel matrix. This method easy allows the exchange of signaling molecules and metabolites that produced by other organisms and cells here enables to grow with nutrient like natural environment due to constant flow of low concentration nutrient further flowcytometer was used to observe cells and metabolites, number of marine less culturable microbes was isolated from this techniques [101-103]. After isolation and detection isolated microbes grow in oligotrophic growth condition with amendment of growth factor to obtain novelsecondary metabolites for drug development.

## Metagenomic approach approaches

Widely accepted concept about microbes, approximately < 1% microbes able to culturable at laboratory scale from envirnmental samples. Metagenomic approach is able to overcome this bottleneck by development of culture independent techniques. It based on DNA sequencing, direct sample DNA analysis and investigate the organisms in evolution point of view. Microbial analysis of water sample from Sargasso sea was investigate by metagenomic approach found far greater sequence then previously investigated [104]. This investigation of microbial biodiversity was open the new horizon in microbial world for development of novelsecondary metabolites from unique sequence by proteomic approach. Thus, this techniques also maximizes the libraries of diversity of marine natural products through direct genomic assessment [105,106]. Isolated DNA sequence from marine sample was digested by restriction enzymes into fragments, further it cloned and tagged with vector transfer into surrogate host organism for protein expression as a secondary metabolites. Such type development of metagenomic libraries from the environmental samples which contain huge number of novel genes that encode for unique beneficial peptide chain of secondary metabolites that have high pharmaceutical value.

## Purification and characterization

Secondary metabolites compounds purification and characterization is important task of obtained compounds from marine derived organisms. In the pharmaceutical industry the importance of both organism and its produce novel bioactive compounds is very high due its efficiency and potency and further develop at laboratory and industry scale. Marine derived natural product has required long time testing and characterization. Marine derived natural products chemistry include isolation of a desired compound, exploit the purification and characterization technologies to get as a ultrapure product compound as possible from of the initial sample and succeeding structural and analytical analysis of the compound using chromatographic, spectroscopic and nuclear magnetic resonance techniques. The input of separation technician to isolate new structure from the marine drive secondary metabolites which can act as starting materials for biochemical synthesis of pure drug. Purified bioactive compounds serve as a lead role in structure elucidation through basic medicinal chemistry or combinatorial synthesis approach to achieve high yield of novel drug with potential activity and less side effects.

## Extraction of secondary metabolites

Marine microbial isolate was inoculated in halophilic broth or Difco™ marine media and placed in a orbital shaker (2000 g) for incubated at 37 °C for 7 days period. After growth observation bacterial

or fungal culture was spun at 5000 g for 50 min and the supernatant filtered through a 0.22 µm membrane filter. The filtrates were extracted with the equal volume of ethyl acetate. Extraction process was repeated for four times and then the extract was concentrated in a rotary evaporator and lyophilized. The crude extracts were stored at 4 °C for further use.

## Purification of crude secondary metabolites

The preparative silica column chromatography (30 cm column length; 50–80 µm packed particle size; 0.5 ml elution flow rate and three bed volume elution) was applied to purify the crude marine derived metabolites. Variable ratio of the mobile phases such as hexane/ethyl acetate and ethyl acetate/methanol were used for eluting the bioactive compounds. The different eluates were collected and concentrated by evaporation in a rotary evaporator and remains extract was stored at 4 °C. Initial characterization by thin liquid chromatography though the fractions were spotted on silica gel plates GF254 (Merck), 20 · 20 cm, 1 mm thick and the chromatogram was developed using, hexane: ethyl acetate (8:2) as mobile phase further dry plate were visualized under UV wavelength.

## Purity and fractionation

Crude organic extract usually contains complex mixtures of many different compounds, thus several sequential purification steps are necessary to obtain specific compound. Usually the desired compound represents less than <1% of the crude organic extract [107]. Purified marine-natural compound is mandatory for structural characterization, and to identify its biological and chemical activities. In some cases, certain structural feature was not detected in a crud extract, which indicates that the extract does not contain the desired compound(s) [108]. Fractionation process mainly conducted through the separation of a crude mixture into a several number of separate fractions. For instance, elute from chromatography column is divided into a feasible number of equal sized portions, and subsequently analyzed these fractions in order to determine the possible compounds of interest [108].

In general, for a compound identification, there are two ways: (a) bioactivity assay, (b) physical assay, which can be done by modern chromatography techniques such as HPLC, TLC, and LC-MS.

**Bioactivity assays:** Typical bioactivity assays of natural metabolites derived from marine microbes are similar to assays of other active metabolites derived from non-marine organisms. Antimicrobial activity screening of bacterial and fungal isolates can be determined by a double layer tech [109], or agar diffusion method [23,110]. The minimum inhibitory concentration (MIC) was identified by serial dilution assay according to the National Committee for Clinical Laboratory (NCCL) [111]. The isolated strains were further tested on the media against five indicators pathogenic microorganisms (i) *M. tetragenus*, (ii) *S. aureus*, (iii) *B. subtilis,* (iv) *E. coli* and (v) *V. anguillarum* [70]. The antimicrobial activity will be calculated by measuring diameters of inhibition zones surrounding the tested organisms. On the other hand, the anticancer activity was determined by using cell culture, MTT assay (cytotoxicity effect)**,** and apoptosis % assay and all of which were described in previous studies [29]. Most of cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fecal calf serum (FCS) and placed it into incubator at 5% $CO_2$ in a 37°C. Furthermore, the MTT assay was used to assess the cytotoxic effect of marine bacterial extracts against different cancer cell lines, in which the cultured cells were treated with the compound (3-(4,5-Dimethylthiazol-2-yl), 5-diphenyltetrazolium bromide). Grown cells at calculated density were treated with marine bacterial extracts, and incubated with the MTT solution, and then microtiter plate reader was used to measure the optical density at 540 (OD). Finally, the obtained results were analyzed using a software or Microsoft office excel. Moreover, the apoptosis assay was conducted in order to count the apoptotic cell deaths %, started by seeding a calculated density of cancer cells in 96-well plates. After incubation, obtained fresh cultured cell lines were treated with different concentrations of marine bacterial extracts, which were diluted in DMEM., Thereafter, the treated cells were incubated at different time intervals, at 37° C, and positive control was treated with $H_2O_2$. After treatment and incubation, cells were lifted and stained with dye and apoptotic cells were determined by high throughput flow cytometer (HTFC) screening system [29]. Similarly, Yi et al. have followed strict criteria in order to explore the signaling pathways of cytotoxic marine-bacterial extracts [112], which include: (i) MTT assay was conducted for cytotoxicity test, (ii) morphological examination after acridine orange (AO) or ethidium bromide (EB) staining (looking for cell shrinkage or nuclei condensation), (iii) DNA fragmentation was detected by labeling of nuclear DNA fragments and gel electrophoresis analysis, and (vi) flow cytometry was used for cell cycle analysis and apoptotic cell death quantification.

**Physical assays:** Physical assays were used to identify marine-natural compounds at molecular level, which include thin-layer chromatography (TLC), Ultra High Performance Liquid Chromatography (HPLC), ion chromatography (IC), High Performance Thin-layer Liquid Chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS), and Gas chromatography-mass spectrometry (GCMS). Most of the characterization based on separation process by chromatography, which involves distribution of a compound between two phases: mobile phase and stationary phase.

Recently, Zhang et al. have characterized a number of marine natural products by using modern chromatography techniques, namely; GC-MS, LC-MS, and IC [113]. Most of microbial metabolites were characterized or identified based on mass charge ratio (m/z), and retention time of ion spectra of GC-MS, and further accurately matched with standard data of library NIST. In addition, HPLC-MS was also used to identify the biodegradation products from metabolites [113,114]. Ion chromatography (IC) was used to determine nitrite, nitrate, formic acid and succinic acid availability in a mixture. Keyhani and Roseman have characterized the intermediate metabolites of chitin catabolic cascade in a marine bacterium, *Vibrio furnissii*. This has been done by using the following seven steps of purification process [115]: i) Crude extract to purify the desired compound, ii) Streptomycin sulphate precipitation, iii) Ammonium sulphate fractionation, iv) DEAE Chromatography, v) Hydroxylapatite column chromatography, vi) Gel filtration chromatography, and vii) HPLC Ion-exchange column chromatography.

**Nature of the active metabolites:** Determining general properties of the molecule can also help to ascertain the compound at early stages. This nature of specific compounds depends on size, solubility, stability, charge and acid/base properties. Solubility mainly depends on hydrophobicity and hydrophilicity nature of the compound, and it is an indicator of the polarity of the desired compound. This can be determined by getting a deride portion of the mixture and re-dissolve it in different solvents with different range of polarities. Suitable solvents include acetonitrile, chloroform, dichloromethane, ethyl acetate, hexane, methanol, petroleum ether and water. Similarly, acid/base properties should be determined by portioning experiments with a wide range of pH level (from 3 to 10). Moreover, charge properties of

the compound can be examined by addition of a different range ion exchanger into the compound mixture. Furthermore, heat stability can be determined by sample incubation at variable temperature and time in water bath, mainly at 80/90° C for at least 10 min, and further check for any physical changes of the sample. Finally, size of the compound can be determined by detecting proteins in either ultrafiltration membrane or dialysis tubing [108].

**Antibiotic from marine organisms**

The explored antibiotics mostly are extracellular secondary metabolites, which are secreted by microorganisms into growing media [116], and further screened out and characterized for drug development for various infectious diseases [117]. Previous studies have shown that actinomycetes are a rich source of antibiotic development, such as peptides or glycopeptides [118,119], tetracycline [120], macrolides [121], angucyclinone [122], phenazines [123], polyenes [124], anthaquinones [125], anthracyclines [126], B-lactams, lactons [127] and many others [128]. Recently, Abdelmohsen et al. have isolated *actinomycetes* from Red sea, and further the crude extracts have demonstrated antibacterial and antiviral activities [43]. In previous study, aminoglycosides have been reported from marine-derived *actinomycetes* with significant antimicrobial activities [128].

**Peptides form Red sea brine water:** Peptides were also isolated and characterized from marine-derived *actinomycetes* with antimicrobial activities [128]. Previous studies have isolated three isoforms of a novel C-terminally amidated peptide from gills of the Red sea bream, *Chrysophrys* (Pagrus) major. The peptide sequences were identified by using a combination of Edman degradation, MS and HPLC analysis of native and synthetic peptides. The three isoforms of peptides, named chrysophsin-1 (25 amino acids), chrysophsin-2 (25 amino acids), and chrysophsin-3 (20 amino acids). The identified isoforms are highly cationic, and containing C-terminal RRRH sequence. In addition, the synthetic peptides have showed a significant bactericidal activity against Gram-positive and Gram-negative bacteria such as *E. coli, B. subtilis*, fish and crustacean pathogens. Recently, Abdelmohsen et al. [129] have reported three different types of biosynthetic genes from 20 *actinomycete* isolates derived from the Red sea, which consist of PKS-I and PKS-II (polyketide synthases), and non-ribosomal peptide synthetases (NRPS) [43]. Each isolate encoded at least one type of biosynthetic gene, and further the crude extracts displayed an antimicrobial activity.

**Enzymes from red sea brine water:** A wide range of halophilic and thermophilic bacterial and archaeal communities harbour the Red sea brine-pools. These marine-derived microorganisms are a potential source of enzymes for pharmaceutical and biotechnology-based applications. Marine enzyme is a protein with a unique nature secreted by marine organisms in hyper saline extreme condition. Enzymes-derived from the Red sea microorganisms exhibit unique properties, and have the capability to be stable at various extreme conditions such as hyper-thermal, hyper saline, and high barophilicity. In most recent study, it was demonstrated that there was a correlation between *actinomycetes* diversity and sediments of mangrove rhizopher age in Jazan coast. The obtained results have showed that *actinomycetes* associated with mangrove are an important source for halotolerant degrading enzymes particularly cellulose and lipase [130]. Previous study has also suggested that the Red sea could be a rich source of enzymes for industrial and biotech-based applications [131].

**Novel metabolites amiability:** Recent study has shown some examples of novel secondary metabolites that have been isolated and characterized from marine *actinomycetes* from 2005 to 2010 [132]. Fotso et al. have elucidated the structures of novel metabolites produced by a marine *Vibrio* sp. derived from the Red sea. The secondary metabolites displayed antibacterial and cytotoxic activities, and their structures were identified by 1D and 2D NMR spectra and by comparison with synthetic material [133].

**Composite drug development with novel metabolites:** Natural compounds or their synthetic derivatives represent over 60% of total active compound according to principals of the pharmaceutical formulations [134]. Previously, FDA also approved that natural products and active metabolites are important sources for exploring novel therapeutic agents and drug development. Some drugs are recently approved as a potential therapeutic compounds derived from marine sources (give examples of these drugs).

**Clinical trial and difficulties:** Currently, FDA approved three new drugs, another drug also registered from EU, 13 marine-derived natural products are in clinical pipeline, and larger portion of marine-derived metabolites are in the preclinical pipeline. There are 13 marine natural compounds in clinical pipeline, which are either in Phase I, Phase II, or Phase III clinical trials. FDA approved drugs from United State Pharmacpeia, includes cytarabine, vidarabine, and ziconotide [99]. Between 1998-2006, the global marine pharmaceuticals-preclinical pipeline has reported 592 marine-derived bio-products with anticancer and cytotoxic activity, and 666 other bio-products with different pharmacological activities (such as antibacterial, antifungal, antiviral, antiprotozoal, and anti-inflammatory activities; actions on cardiovascular, endocrine, immune and nervous systems), for more information web:http://marinepharmacology.midwestern.edu/

**Conclusion and future prospect:** In conclusion, only few studies have been done to explore the microbial diversity and their biological activities of the Red sea. Therefore, further studies need to be conducted at different geographical locations and different depths of the Red sea to explore more novel marine natural products. Further investigations are necessary to determine the biological and chemical diversity, to identify and purify the crude organic extracts of the existed marine microorganisms derived from the Red sea. In this review article, we have illustrated the previously reported microbial diversity of the Red sea with their antibacterial and/or anticancer activities. Continued development of improved cultivation techniques and molecular technologies for accessing the marine environment provide an access to significant new sources of more marine-derived natural products, which is important for development of new and potent therapeutic agents for various infectious diseases.

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