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PHARMACEUTICAL POTENTIAL OF ENDOPHYTES ASSOCIATED TO MARINE SPONGE AND ALGAE FROM THE BAY OF BENGAL AND THEIR CONTRIBUTION TO THE BLUE ECONOMY OF BANGLADESH

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ABSTRACT: Bangladesh is gifted with the world's largest bay, rich in untapped resources that are undeniably beneficial to human wellbeing and the national economy. However, the people of Bangladesh have not yet been capable of using the vast marine resources of the Bay of Bengal due to a lack of adequate funds and technological limitations. On the other hand, countries like Japan, China and Indonesia utilize their resources from the sea and develop therapeutic agents and molecules to produce efficient lifesaving drugs. Nowadays, marine habitat is considered one of the most resourceful areas for natural product research. Marine organisms possess enormous biological, biochemical, and biosynthetic potential. Likewise, bioactive compounds from marine microorganisms opened the door for novel therapeutic compounds discovery and development, *i.e.,* anti-cancer, antibiotics, antifungals, and antiparasitic. Marine endophytes from algae and sponges are almost endless sources of novel compounds with numerous potential therapeutic applications due to their enormous diversity and intrinsic ability to create natural products of medical and pharmaceutical significance. There are nearly 257 pharmaceutical companies in Bangladesh fulfilling national demand. But these pharmaceutical industries are still depending on raw materials imported from abroad to manufacture essential drugs, which raises the manufacturing cost. Therefore, Bangladesh needs to strengthen its capacity to research marine resources, collect, isolate, analyze and characterize potential compounds to utilize them in healthcare sectors. Such a systematic approach to isolate potential pharmaceutical compounds from marine endophytes will help achieve the country's sustainable development goal and will greatly contribute to the blue economy.

INTRODUCTION: Oceans play a crucial role for world people directly and indirectly. Approximately 70% of the earth is surrounded by seawater.

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Oceans contain uncountable numbers of living microorganisms widely distributed in various hostile habitats indicating the presence of complex secondary metabolites, novel enzymes, a huge number of bioactive chemical compounds, proteins categorized into different functional groups benzopyranones, chinones, flavonoids, quinones, steroids, saponins, tannins, terpenoids, alkaloids, phenolic acids, *etc.* [1, 8]. Microorganisms are found almost everywhere, especially endophytic fungi that live in plant species. However, Marine fungi

are present in various substances such as sponges, fish, algae, and mangroves, producing bioactive and metabolic compounds. Marine fungi are highly sensitive and found in particular living conditions such as pH, temperature, salinity, current, and nutrition. Presently, many drugs resistant to certain diseases encourage more advanced versions of drugs to act properly; in that case, marine fungi can be used to develop new drugs for non-communicable and various infectious diseases [2]. Endophyte fungi found the considerable activity of bioactive compounds such as anti-cancer, antitumor, and antioxidant has a potential application in pharmaceutical goods or medicines, aquaculture, food, and industrial sectors [3]. There are many biodiversities in the ocean, the most notable of which are algae, sponge, seaweed, fungi, and a marine sponge that depends on a suitable environment, especially a rocky shore. Marine sponge-associated endophytes have novel therapeutic agents such as free radical scavenging, anti-inflammatory, and cytotoxic activity [4].

Again, secondary metabolites from endophytes associated with marine algae, sponge, seaweed, fungi, and sponge are very diverse in their function, ranging from antiviral, antifungal, immunosuppressive, anti-inflammatory antitumor, and many other functions of biotechnological and pharmaceutical significance [5]. Many marine microorganisms are available such as sponge, algae, coral, and endophytic fungi potential application in mortal anti-cancer drugs (tyrosinase, methioninase taxol, L-asparaginase, and L-glutaminase), antifungal agents (prodigiosin and saadamycin), and antiviral agents. The ecosystem plays a significant role in the mangrove forest and contains approximately 300 species of leafy *Rhizophora mucronata* trees and 350 fungal endophytes, some of the other deciduous [6]. Fungal endophyte has some antibacterial activity and anti-cancer activity such as cervical cancer (HeLa), lung cancer (A549) and skin cancer (A431) breast cancer (MCF-7) [7].

Endophytes from marine origin, at present, raise great interest in research fellows; since the government is seeking to promote blue-economy and opting for exploring and assessing our oceanic resources by developing research institutes and state-of-the-art laboratories, it is time for the qualified manpower to set the mind in discovering new functional drugs from marine resources to make an impact in the pharmaceutical industry, a multibillion prospect for Bangladesh as well as other sectors yet to discover.

Endophytes: Endophytes are microorganisms, often known to be fungi and bacteria, residing inside the marine seaweed tissue without causing harm to the host with external symptoms. Generation and secretion of biologically active compounds may take place in some endophytes. Those are known as secondary metabolites, which can assist the host seaweed in fighting against bacterial, fungal, and other pest pathogens. Endophytes are collected from the plants living on the sea, like green algae [9]. Endophytic fungi, which live in the host's living tissues, are found in almost every organism. These microorganisms establish a variety of relationships with the host ranging from symbiotic to pathogenic [10]. Marine Endophytes are an extensively new source of pharmacologically relevant compounds due to their species richness and diversity.

Endophytes Associated to Marine Sponge: In the marine environment, sponges harboring several endophytes are valuable sources of many unique bioactive natural products, contributing to nearly 30% of all-natural products discovered from marine sources [11, 12]. The microbial system of sponges serves as a source of potential genetic resources and secondary metabolites that raise great interest in genomics, biochemical, pharmaceuticals, antimicrobials, biota exploitation, *etc* [11].

Marine sponges are well known to host a large community of endophytes, which constitute an important percentage (up to 50–60%) of the host biomass[11]. Previous studies have found more than 15,000 natural bioactive compounds, *i.e.*, antibiotics from marine invertebrates. More than 300 new and novel bioactive compounds were recently discovered from a single phylum, Porifera, and subjected to preclinical and clinical trials [5].

Various types of endophytes producing bioactive compounds have been found from the marine environment associated with the sponge, which can play a vital role in producing the valuable drug in the modern world [4].

Marine Sponge Associated Endophytic Fungi: Endophytic fungi are one such group that has been reported to be a vital and invaluable source of novel therapeutic agents possessing several bioactive properties, including free radical scavenging activity, neuritogenic activity, anti-cancer activity, kinase inhibition, *etc*. [4]. Studies have found that marine sponge-associated fungi produce structurally unique bioactive compounds, which are an excellent and promising source of potent secondary metabolites with anti-inflammatory activities. Also, it has been reported that several secondary metabolites such as alkaloids, terpenoids, xanthones, sterols, diphenyl ether and anthraquinones with potent anti-cancer properties are isolated from *Aspergillus sp*. [4]. **Table 1** shows some sponge-associated endophytic fungi that has been isolated from the Arabian sea.

TABLE 1: LIST OF SPONGE-ASSOCIATED ENDOPHYTIC FUNGI THAT HAS BEEN FOUND FROM ARABIAN SEA

Sl. no.	Sponge	Associated Fungi
1	*Callyspongia fibrosa*	*Aspergillus tamarii* MCCF 102; *Aspergillus sp*. MCCF 103; *Lasiodiplodia sp*. MCCF 104 *Aspergillus sp*.; MCCF 105 *Penicillium sp.* MCCF 106 *Schizophyllum sp*.; MCCF 107 *Aspergillus sp.;* MCCF 108 *Penicillium sp.* MCCF 109
2	*Tedania anhelans*	*Penicillium sp*. MCCF 110 *Aspergillus sp*. MCCF 111 *Aspergillus sp*. MCCF 112 *Penicillium sp*. MCCF 113 *Aspergillus sp.* MCCF 114 *Penicillium sp.* MCCF 115 *Penicillium sp*. MCCF 116 *Penicillium sp.* MCCF 117 *Monascus sp*. MCCF 118
3	*Myxilla arenaria*	*Aspergillus sp.* MCCF 119 *Aspergillus sp*. MCCF 120

The isolation and characterization of lead compounds responsible for these activities need to be further investigated.

Marine Sponge Associated Endophytic Bacteria: Marine sponge's shelter diversified bacterial symbionts, many of which produce a wide range of bioactive compounds, including antibiotics that protect the host against different pathogens. Notably, most sponge-based antibiotics have been derived from bacteria belonging to *Actinobacteria* [11, 5]. The marine bacteria associated with sponges can produce more antibiotic substances. Extensive research has been done to explore the foreseeing potential of marine microbes, which are reported to have some unique pharmaceutically essential properties. In Bangladesh, the Saint Martin's Island area of the Bay of Bengal is rich in organisms. Recently a total of 645 bacterial isolates have been isolated from nine sponges that colonized that area. In this connection, fifteen bacterial genera have been identified based on the phenotypic characteristics **Table 2.**

TABLE 2: LIST OF BACTERIA SPECIES ISOLATED FROM SAINT MARTIN'S ISLAND AREA OF THE BAY OF BENGAL

Vibrio sp.	*Marinobacterium sp.*	*Bacillus sp.*
Shewanella sp.	*Chromohalobacter sp.*	*Staphylococcus sp.*
Photobacterium sp.	*Thalassolituus sp.*	*Halobacillus sp.*
Salinicola sp.	*Burkholderia sp.*	*Thalassobacillus sp.*
Providencia sp.	*Oceanobacillus sp.*	*Sediminibacillus sp.*

They show antibacterial activity very prospective for pharmaceuticals to produce antibiotics. Two promising strains of *B. subtilis* have been detected in recent studies which are isolated from the sponge- *H. rosea* and *H. columella* respectively that exhibit inhibition against high virulent strains of *A. veronii* [11]. Bioactive metabolites found in *Vibrio sp*. EA348 has great biotechnological and medicinal significance because of antibacterial, antifungal, and plant growth [5]. Various *Vibrio spp*. are commonly distributed in the marine environment, some of which are pathogenic while others are beneficial and considered potential sources of novel bioactive natural products. The study has found that the highest species diversity is observed among the Bacillus sp. followed by *Vibrio sp*., *Oceanobacillus sp*., and Staphylococcus sp. Marine Bacillus strains are well known for producing structurally diverse bioactive compounds with potential antibiotic activity [11].

The diversity and biological activity of the bacteria have been found in the sponge *Pione vastifica* and *Siphonochalina siphonella* collected from the Jeddah, Red Sea. A study has also been done on the diversity in *Suberea mollis* sponge-associated bacteria from the Arabian Sea [11]. A previous study has found that 35 sponge-associated bacterial genera belong to the phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes,* and *Cyanbacteria* produce antimicrobial compounds. Among these, 9% of the currently known antimicrobials active

against viruses, bacteria, and fungi are produced by *Bacillus spp*. It is reported that Bacilli produce antibiotics such as bacillin, bacitracin, subtilin, difficidin, polymyxin, oxydifficidin, gramicidin, mycobacillin, or bacildxlomycin B.[4]

Endophytes Associated to Marine Algae: Marine algae are considered to be one of the most potential resources in the ocean. Marine algae are usually classified into three groups brown seaweed (Phaeophyta), green seaweed (Chlorophyta), and red seaweeds (Rhodophyta), which are based on their thallus colour derived from natural chlorophylls and pigments [12]. Algae in marine habitats adapt to frequent and occasional environmental changes such as high salinity, low oxygen content, nutrient limitation, excessively high light, and drought, which may trigger endophytes to produce specific bioactive secondary metabolites that participate in the defense mechanisms of the hosts for wide adjustment with the changing situation [13] .

Algae Associated Endophytic Fungi: A total of 182 metabolites have been discovered from marine algae-associated endophytic fungi in the past decades (mainly from 2002 to mid-2015). A variety of novel compounds have been isolated and discovered, exhibiting anti-cancer, antibiotic, antiviral anti-oxidative, and kinase inhibitory or activated activity among their biological features. [13]. Endophytic fungi residing in marine algae are chemical synthesizers and ubiquitous organisms living intercellular or intracellular.

The anti-cancer and antibacterial activities of the endophytic fungi, *P. chrysogenum* collected from the marine algae, *C. antennina* have been investigated presently. The Cha EA extract inhibited the HeLa cells, and the apoptotic cell death has been observed by the live dead cell assay using PI and AO/PI staining [10]. Thus, the importance and utility of endophytic fungi associated with Marine algae are immense in the field of blue-biotechnology, pharmaceuticals and therapeutic advancement, and other fields still unexplored.

Algae Associated Endophytic Bacteria: Algae-associated endophytic bacteria are one of the abundant resources of the ocean. Previous studies done on seaweed surface of Todos Santos Bay, B.C. have found that algae-derived endophytic bacteria of the family Firmicutes, *Proteobacteria* and *Actinobacteria* produce compounds capable of slowing down the growth of HCT-116 colorectal cancer cells. Also, it was found that the bacteria Microbulbifer thermotolerans and *Pseudoal-teromonas sp*, are able to produce biofilms and chemical compounds that guard them against the other protozoans. *Bacillus sp*. species have been found to possess chemical compounds with anti-cancer activity [14]. **Table 3** compiles some examples of bioactive bacterial strains and diseases that can be tackled using secondary metabolites from various bacteria. These microorganisms are important as an ideal source of bioactive compounds [14].

TABLE 3: LIST OF BACTERIA STRAINS PRODUCING BIOACTIVE COMPOUNDS AND THEIR THERAPEUTIC APPLICATIONS

Bacteria	Activity	Disease
Pseudomonas bromoutilis	Anticancer	Pneumonia, osteitis, arthritis, endocarditis, localized abscesses
Chromobacteria marinum	Antibacterial	Pneumonia, osteitis, arthritis, endocarditis, localized abscesses
Flavobacteria uliginosum	Anticancer	Viral tumor
Bacillus sp.	Anticancer	Colorectal Cancer
Lactococcus lactis	Anticancer	Cervical Cancer
Staphylococcus aureoverticillatus	Anticancer	Tumors
Marinobacter hydrocarbonoclasticus	Antibacterial (Siderofore)	Tuberculosis, carbuncle (anthrax like)

Pharmacological Value: Since the primitive age, drugs made of direct natural organisms have been used to heal specific diseases. Later on, science and its discovery regarding chemical molecules and compounds previously known as natural medicine have contributed to modern medicinal advancement. According to the World Health Organization (WHO), about 60% of the world's population rely on traditional medicine for their health care. As such, natural products, as it claims,

provide vital clues in drug discovery and are therefore considered the cornerstone of drug development. Many drugs available in the market today were discovered from natural sources [15, 8]. Marine organisms have always been the most desired source for pharmaceuticals due to their anti-inflammatory, anti-cancer, antibacterial and neuroprotective properties, and they are the most potent candidates to cure acute and chronic disorders. Out of 13,000 molecules tested for their pharmacological efficacy, 3000 molecules have the potential to act as an active drug [18].

A study has found that the isolates derived from the sponge *H. erectus* produce different bioactive secondary metabolites such as hydrolases (alkaline protease, cellulase, L-glutaminase, laccase, xylanase, lipase, and L-asparaginase), enzyme inhibitors (inhibitors for α-amylase, α-glucosidase, β-glucosidase, βglucuronidase, and tyrosinase), antibacterial, antifungal, antiviral (against HCV), and antiproliferative activities (against HepG2 and Caco2 cell lines). Proteases have a significant role in many physiological and pathological processes such as protein catabolism, blood coagulation, cell growth, migration, inflammation, tumor growth, and metastasis [15]. Based on this study, endophytes of sponges are considered the apt sources for producing Applied Biochemistry and Biotechnology diverse and multiple biologically active compounds of potential pharmaceutical and industrial applications. Marine-derived endophytic fungi, as well as other bioactive compounds, have been the focus of interest which have led to important drugs available from the pharmaceutical industry such as Prialt®, Yondelis®, Aplidin®, and Irvalec®. Some drugs are under clinical development, and a few have been approved as potential cancer drugs, namely cytarabin Ara-C, Yondelis®, and trabectedin [16].

Sponges are the most well-studied marine invertebrates with potential medicinal chemistry applications, and their chemical defense systems make them a good starting point for new drug leads. Sponge alone produces around 3300 antibiotics and other bioactive chemicals [17]. In the case of new drug candidates or drug development, the endophytic fungi from marine algae can be an innovative and potential source. Hence, a structured plan for using and preserving those species is highly required [17]. From the economic point of view, marine algae represent an essential resource of food and industrial raw materials. The Caribbean Sea coast of Colombia is home to a plethora of economically valuable species that are utilized as human food, medical goods, fertilizers, and fuel and play a key part in the extraction of phycocolloids and hydrocolloids. Despite discussion about seaweed's potential as a direct source of proteins and pharmaceuticals, demand for phycocolloids will be the driving force in the future development of the marine algae world resources[14].

Blue Economy potential of Marine Endophytes for Bangladesh: Bangladesh is a developing country with immense prospects in different sectors. Due to the large population, Bangladesh faces problems every day like unemployment, infectious disease, high cost of treatment and challenges of vaccination leadings, genetic disease, drug resistance, inability to meet basic needs, *etc*. So, identifying new sectors to meet those limitations is now the demand of time. Here utilizing marine resources can be a potential and significant way to conquer our challenges. Fortunately, it is reported that more than 20,000 Marine novel products have been discovered from marine microorganisms [19]. Bangladeshi Pharmaceutical industries play an important role in the economic sector, and right now, it is the second-largest economy contributing to the GDP growth [20]. The demand for food products and new drugs is increasing in line with the increase in the global population. The Pharmaceutical sector needs highly qualified human resources modern lab with the most advanced technology.

According to the Bangladesh Association of Pharmaceutical Industries (BAPI) and Directorate General of Drug Administration (DGDA), 257 pharmaceutical companies in Bangladesh are working, and 150 are licensed. Some specialized drugs such as vaccines, anti-cancer drugs and hormone drugs are imported to meet 2% of the demand.

Approximately 98.0% of total demand are manufacturing, 80% of generic drugs are produced in Bangladesh, the rest 18% are patented drugs, and the rest 2.0% are imported. There are opportunities for the pharmaceutical industries in Bangladesh,

and next, this industry will turn into a multi-billion-dollar economy if our marine resources are utilized properly. Bangladesh has *exported* pharmaceutical products worth USD 130.0 million in 2018-19 [21, 22, 23].

CONCLUSION: Bangladesh is a lower-middle-income country with a population of about 164 million. This huge population suffers from many life-threatening disorders and spends enormous money on effective remedies. Although the pharmaceutical industries manufacturing of Bangladesh is manufacturing many lifesaving drugs, most of the drugs are patented in some other countries and the number of R and D drugs is almost NIL. As a result, these pharmaceutical companies spend millions of dollars to import raw materials to manufacture drugs. Since the per capita income of the Bangladeshi population is increasing every year, Bangladesh will no longer have access to a special World Trade Organization (WTO) waiver, which exempts the industry from the Agreement on Trade-Related Aspects of International Property Rights (TRIPS) status of manufacturing low-cost generic drugs. Therefore, the time has come to explore the vast natural resources of the Bay of Bengal and take effective scientific measures to meet future demand. Bangladesh has adequate marine resources considering a huge bay lying backyard. The marine environment is presently well investigated as one of the fundamental sources regarding natural products in research since organisms from oceans have exhibited exceptional biological, biochemical, and biosynthetic potential. We need to discover these resources to fulfill our national demand and find other uses in human lives, reducing pressure on other resources. To meet the challenge of the modern era, utilizing these resources will play a crucial role for human wellbeing and contribute to the sustainable development of the blue economy in Bangladesh, and may pave the way of supplying cheap and effective medication for the people of the Indian subcontinent.

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