# Review On - Therapeutic Efficacy of Secondary Metabolites Extracted from Marine Algae Associated Endophytic Fungi

**Dipika Madhukar Magdum**

Department of Biotechnology, REVA University, Kattigenhalli, Yelahanka, Bengaluru-560064, India

**Abstract:** ***Drug resistant are high in country especially cancer and infected diseases therefore, there is a need to search new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture, and industrial applications. Cancer is a major health problem due to its high rate of morbidity and mortality. Severe side effects are associated with most of the available anti-cancer drugs; hence we are focusing on natural compounds with minimal toxicity. Endophytic fungi that are residing a symptomatically in internal tissues of all higher plants are of growing interest as promising sources of biologically and pharmacologically active agents and bio-active molecules. Especially marine endophytic fungi have the ability to synthesize novel metabolites due to its high salinity, high stress and unexplored marine environmental status. The marine environment is home to an immensely vast and complex array of species and ecosystems, most of which remain undiscovered. This does not come as a surprise, considering that the ocean covers approximately 70% of the planet's surface. Enumerate a total of approximately 226.000 described marine eukaryotic species, and estimate that one-third to two-thirds of marine species are yet to describe. Natural compounds are the main source of active ingredients in medicines, Indeed, the discovery of natural products in terrestrial fauna and flora has produced a grand diversity of bioactive compounds with the most varied chemistry and effects, as diverse as anticancer, anti-inflammatory, anti-parasitic, antiviral, analgesic, immuno-modulator, anti-diabetic activity, among many others.***

**Keywords:** Endophytic fungi, Secondary metabolites, Bioactive products

## 1. Introduction

The marine environment is distinguished by unique groups of organisms such as animals, plants and microbes. The enormous biodiversity of marine habitats found to be plants and microbes. Plant has variety of diversity in the form of macro algae, sea grass and mangrove. Algae (macroalgae, seaweed) are represented by at least 30,000 species worldwide supplying oxygen to the biosphere, food for fish and man, medicine and fertilizers as well as being a prolific source of structurally unique natural product. Marine environment provides a unique and challenging ecological niche enabling the production of a plethora of structurally diverse secondary metabolites from the inhabiting organisms.Algae inhabiting marine ecosystems adapt to frequent and sporadic environmental changes such as high salinity, low oxygen content, nutrient limitation, excessively high light, and drought, which may stress endophytes to produce certain bioactive secondary metabolites to participate in the defense mechanisms of the hosts. The first record of algae being used in Western medicine to treat breast cancer dates back to the 1960's when "algasol T331" was introduced in Italy (Claudio and Stendardo 1965; Fujihara*et al*,. 1984). Algae and sea grasses were amongst the first organisms investigated by marine natural products scientists on their quest for novel pharmaceutical compounds (Hirschfield *et al*., 1973: Scheuer 1973:Fenical and Paul 1984; Fujiharaet al.,1984;Joseph and Baker 1984;).Endophytic fungi usually live inside plant tissues without causing any noticeable symptoms of disease (Arnold 2007; Sieber 2007). Because of the unique living environment, they are capable of synthesizing special bioactive compounds and may establish close relationships with the host plant cell. The plants are considered to provide nutrients to the microbes; while the microbe, may in some degree, promote host growth and increase host fitness in severe environments, or protect the host from insect pests (Azevedo *et al.*, 2000; Gao *et al.*, 2005) as well as fungal pathogens (Mejia *et al.*, 2008; You *et al.*, 2009), and possibly play a role in litter degradation (Sun *et al.*, 2011). Thus, the endophytic fungi as a potential resource of bioactive metabolites have been received extensive attention in recent years. In general, the studies concerning endophytic fungi, at present, are mainly focused on terrestrial plants (Hu *et al.*, 2007; Wei *et al.*, 2007; Rajagopal *et al.*, 2012), but recently, some researchers have turned to so far less investigated aquatic vegetation, such as mangrove forests, algae and sponges (Raghukumar 2008). Algae constitute the major photosynthetic populations and play important roles in both marine and freshwater ecosystems. In recent years, some researchers have isolated several endophytic fungi from multicelluar macroalgae using a standard procedure (Wang *et al.*, 2006; Flewelling *et al.*, 2012), including green algae like Ulva pertusa (Osterhage *et al.*, 2000; Cui *et al.*, 2010), brown algae e.g. Sargassum kjellmanianum, Sargassum tortile, Colpomenia sinuosa, Sargassum thunbergii. (Yamada *et al.*, 2002; Zhang *et al.*, 2007; Du *et al.*, 2012; Sun *et al.*, 2012)

### 1) Fungi-producers of biologically active metabolite

More than 20,000 bioactive metabolites are of microbial origin (Bérdy 2005). Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications (Kock et al., 2001, Bode et al., 2002, Donadio et al., 2002, Chin et al., 2006, Gunatilaka 2006, Mitchell et al., 2008, Stadler & Keller 2008). The success of several medicinal drugs from microbial origin such as the antibiotic penicillin from *Penicillium* sp., the immunosuppressant cyclosporine from *Tolypocladium inflatum* and *Cylindrocarpon lucidum*, the antifungal agent griseofulvin from *Penicillium griseofulvum* fungus, the cholesterol biosynthesis inhibitor lovastatin from *Aspergillus terreus* fungus, and $\beta$-lactam antibiotics from various fungal taxa, has shifted the focus of drug discovery from plants to microorganisms. Taxol (Fig.1), compound is the world's first billion dollar anticancer drug, and it is used for treatment of ovarian and breast cancers, but now it is used to treat a number of other human tissue-proliferating diseases as well. This compound is highly bioactive isolated from *Taxus brevifolia* (Suffness 1995, Wani et al., 1971).

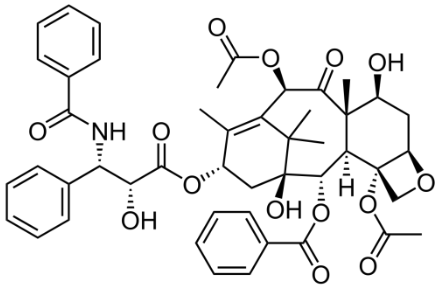


**Figure 1:** Taxol, anti-cancer drug from endophytic fungi

### 2) Secondary metabolites

Plants produce a high diversity of natural products or secondary metabolites which are important for the communication of plants with other organisms. A prominent function is the protection against herbivores and/or microbial pathogens. Some natural products are also involved in defence against abiotic stress, e.g. UV-B exposure. Many of the secondary metabolites have interesting biological properties and quite a number are of medicinal importance. Because the production of the valuable natural products, such as the anticancer drugs like paclitaxel, vinblastine , camptothecin, etc in plants is a costly process, biotechnological alternatives to produce these secondary metabolites more economically become increasingly important. Secondary metabolites from natural sources have made a significant contribution to medicine for millennia. In modern medicine, drugs developed from natural products have been used to treat infectious diseases, cancer, hypertension, and inflammation. Research on immunomodulators for application in vaccines has been sporadic, but it stands to reason that the field could better exploit the biodiversity of active compounds from natural sources. Most new chemical entities (NCE) have been inspired from plants, while microbes have also yielded a significant number of drugs.Increasingly, there are reports of NCE derived from fungi and marine sources, and animals. Although immunopotentiators mined from plants are well established, other organisms have also been evaluated. Advancement in macromolecule separation and spectroscopic techniques essentially brought usage of plant derived secondary metabolites into the limelight for human usage. Currently manyproducts that are used on day to- day basis like dyes, polymers, fibres, glues, oils, flavoring agents and drugs have been derived from natural sources. From an ecological perspective they play significant roles in protection against herbivores, microbial infection and also act as attractants for pollinators, seed dispersing animals, alleopathic agents (Croteau *et al.*, 2000). The specific role of secondary metabolites in organisms defense may involve deterrence or anti-feedant activity and toxicity or acting as precursors to physical defense system. Secondary metabolites thus function as: competitive weapons used against other bacteria, fungi, amoebae, plants, insects, and large animals; (ii) metal transporting agents (iii) agents of symbiosis between microbes and plants, nematodes, insects, and higher animals (iv) sexual hormones and (v) differentiation effectors. Secondary metabolites may be either produced in certain cells, or constitutively expressed by all cells, but in both cases require chemical activation. Eventually, they were exploited for drug, cosmetics and several other industries.

### 3) Classification of secondary metabolites

Secondary metabolites can be classified as Terpenoids, Phenolics and Nitrogen containing compounds. A brief description of each class of molecule is given below.

#### a) Terpenoids

Terpenoids have a wide biological function, and are also used for medicinal purposes. A five-carbon isoprene units, serve as building blocks for these terpenoids. Terpenoids are classified based on the number of isoprene units as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes and polyterpenes. Hemiterpene is a basic isoprene unit and is generally produced as a byproduct of photosynthesis. Monoterpenes are produced by fusion of two isoprenoid units and they are generally available in nature as floral scents. Sesquiterpenes composed of three isopreoid units that are found in numerous essential oils and these are also involved in anti-pathogenic activities. Diterpenes include four isopreoid units and the notable ones include gibberellin hormones and phytoalexins. Tetraterpenes are composed of eight isoprene units. Terpenoids, that contains more than eight- isoprene units are generally classified as polyterpenoids (Bohlmann., 1998; Croteau *et al.*, 2000).

#### b) Phenolics

Phenolics are aromatic compounds characterized by a phenol ring attached to a hydroxyl group. The chemical structures of phenolic compounds vary greatly from simple phenolics (C6) such as hydrobenzoic acid derivatives and catechols to long chain polymers with high molecular weight such as lignins (C6-C3)n and condensed tannins (C6-C3-C6)n. Phenolics are derived from phenyl propanoid acetate pathways. Most phenolic compounds function as structural entities in plants while a few have a role in plant defense. Flavanoid and lignin are the important class of phenols. Flavanoids include more than 4500 different compounds (Croteau *et al.*, 2000).

#### c) Nitrogen containing compounds

This group comprises of a diverse group of chemical with nitrogen in their structure. Most of these molecules have different functionalities and can be classified as follows.

#### d) Alkaloids

Alkaloids are naturally occurring compounds containing basic nitrogen atoms. Most of them contain a heterocyclic ring that has a nitrogen atom. They derive their name from word "Alkaline" that is attributed to any nitrogen-containing base. Alkaloids are produced by wide varieties of organisms like bacteria, fungi, plants and animals. Alkaloids exhibit a wide range of pharmacological properties namely anti-cancer (Eg. Vincristine and Vinblastine), anesthetic and stimulant (Eg. Cocaine, nicotine) and anti-malarial drug (Eg. Quinine) (Tarek, 2012).

- **Terpenoids Indole Alkaloids**
  It is perhaps one of the major groups of alkaloids in the plant kingdom, which comprise of more than 3000 recognised alkaloids till date. Interestingly they are found in eight different natural orders of which the Apocynaceae, the Loganiaceae, and the Rubiaceae are predominantly the best-known sources. The metabolites seem to be involved in plant defense processes, which have enabled the survivability of this long-lived plant genes. (Rischer *et al.*, 2006).
- **Amines**
  Amines are derivatives of ammonia where alkyl or aryl group has replaced one or more hydrogens. They may respectively be called alkylamines and arylamines. Important amines in a biological system include amino acids and biogenic amines. Amino acids are basic building units of proteins and whereas biogenic amine is a biogenic substance with one or more amine groups. They are basic nitrogenous compounds formed mainly by decarboxylation of amino acids. Biogenic amines are organic bases with low molecular weight and are synthesized by microbial, vegetable and animal metabolisms. They include monoamines such as histamine, norepinephrine, epinephrine which function as neurotransmittors and polyamines such as caderverine, putrecine and spermidine function in biosynthesis of tropane alkaloids (Halasz, 1994).

### e) Endophytes

The term endophytes refer to microorganism that reside inside 'endo' the plant tissue 'phyte'(Bacon *et al.*, 2000). These microorganism reside within all plants without causing any visible manifestations of disease condition. They either spend their entire or part of the life cycle inside the host plant.

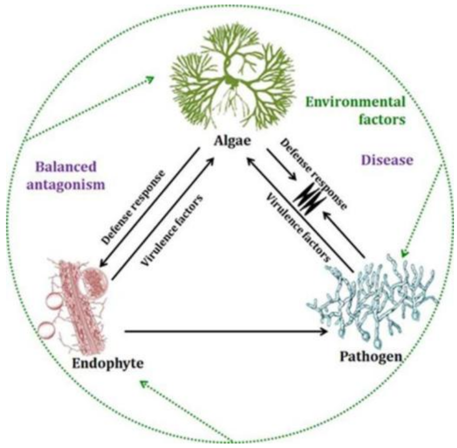


**Figure 2:** Asymptomatic colonization of endophytes.(source: Marine Algicolous Endophytic Fungi - A Promising Drug Resource of the Era, Manomi Sarasan)

### f) Endophytic fungi with therapeutic value

Endophytic colonization within the host tissue confers environmental survival fitness to the plant by combatting there biotic and abiotic stress. At first it was discovered that endophytic inhabitant plant species are known to produce antimicrobial agents that are not originated from the plant. Later it was identified that the microbes that inhabit these plants, in-order to facilitate the plant to fight against the pathogens produce these metabolites after the discovery of taxol producing endophytes from *Taxusbrevifolia*. Several endophytes produce both host specific and host non-specific metabolites. It is therefore well accepted that endophytes even today remain as an untapped potential for production of several secondary metabolites (Strobel *et al.*, 2004; Staneik *et al.*, 2008). These secondary metabolites producing endophytic fungus are known to have multiple roles when exploited by humans. They are reported to have a range of function having anti-diabetic, anti-inflammatory, anticancer, anti-bacterial and so on.

**Table 1:** Examples of compounds isolated from marine sponge-associated fungi and their bioactivity.

| Bioactivity | Fungus | Sponge | Metabolite | Reference |
|---|---|---|---|---|
| Anti-bacterial | *Aspergillus sp.* | *Xestospongia testudinaria* | (-)-Sydonic acid | (D. Li *et al.*, 2012) |
| | *Aspergillus sp.* | *Tethya aurantium* | Austalide R | (Zhou *et al.*, 2014) |
| | *Exophiala sp.* | *Halichondria panacea* | Chlorohydroaspyrones A and B | (D. Zhang, Yang, Kang, Choi, & Son, 2008) |
| Anti-fungal | *Penicillium spp.* | *Tethya aurantium* | Nortryptoquivalin | (Wiese, Ohlendorf, Blümel, Schmaljohann, & Imhoff, 2011) |
| | *Aspergillus insuetus* | *Psammocinia sp.* | Insuetolides A, B and C | (E. Cohen *et al.*, 2011) |
| Bioactivity | Fungus | Sponge | Metabolite | Reference |
| Cytotoxic | *Clonostachys sp.* | Unidentified sponge | IB-01212 | (Cruz *et al.*, 2006) |

### g) Marine system as a source of natural bioactive compounds

The marine environment is home to an immensely vast and complex array of species and ecosystems, most of which remain undiscovered. This does not come as a surprise, considering that the ocean covers approximately 70% of the planet's surface. This massive body of water encompasses different ecological niches, some of which are highly productive and prosperous in biodiversity, such as the sea-land interface and deep ocean thermal vent communities, others, such as the vast open ocean waters, possess limited production and are poor in biomass and diversity. Appeltans *et al.*, (2012) enumerate a total of approximately 226.000 described marine eukaryotic species, and estimate that one-third to two-thirds of marine species are yet to describe (Appeltans *et al.*, 2012). The quest for novel compounds of natural origin has been a persistent ambition for pharmaceutical research. Natural compounds are the main source of active ingredients in medicines, and in spite of modern pharmaceutical synthesis techniques, natural products are still the basis of almost half of all approved drugs. This success is in part due to the fact that natural products usually display high bioavailability, high affinity to target, as well as a minor loss of entropy when binding to proteins (Harvey, 2008). Indeed, the discovery of natural products in terrestrial fauna and flora has produced a grand diversity of bioactive compounds with the most varied chemistry and effects, as diverse as anticancer, anti-inflammatory, anti-parasitic, antiviral, analgesic, immunomodulator, anti-diabetic activity, amongst many others (Newman & Cragg, 2012). The focus is now turning also towards to the marine environment, where the broad and yet to explore biodiversity make promise of new chemical structures. In spite of this interest, the quest for marine natural products poses several challenges which limit its expansion; large and complex molecules, enhanced costs to collect and manipulate species, difficult culturability in laboratory conditions, the lack of technological tools and innovations and also environmental concerns (Bhatnagar & Kim, 2010).

**h) Bioactive compounds and its therapeutic efficacy**

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Iwamoto *et al.*, 1998; Iwamoto *et al.*, 1999; Iwamoto *et al.*, 2001).

Algae are very simple chlorophyll-containing organisms (Bold and Wynne, 1985) composed of one cell or grouped together in colonies or as organisms with many cells, sometimes collaborating together as simple tissues. They vary greatly in size – unicellular of 3–10μm (microns) to giant kelps up to 70cm long and growing at up to 50cm per day (Hillison, 1977). Algae are found everywhere on earth: in the sea, rivers and lakes, on soil and walls, in animal and plants (as symbionts-partners collaborating together); in fact just about everywhere where there is a light to carry out photosynthesis.

Algae can be classified into two main groups; first one is the microalgae, which includes blue green algae, dinoflagellates, bacillariophyta (diatoms)… etc., and second one is macroalgae (seaweeds) which includes green, brown and red algae. (Biological importance of marine algaeAli A.El Gamal).

The term endophytes refer to microorganism that reside inside ‘endo’ the plant tissue ‘phyte’ (Bacon *et al.*, 2000).

Endophytes are microorganisms which live in the internal tissues of other organisms without causing any negative side effects to their host (Strobel & Daisy 2003).They either spend their entire or part of the life cycle inside the host plant. Endophytes diversity are well describe by Hardoim *et al.*, (2015) to be divided into prokaryotic endophytes spanning 4 bacterial phyla, and eukaryotic endophytes consisting of 3 major division in the fungal kingdom (Glomeromycota, Ascomycota and Basidiomycota).

The endophytes contribute to the survival of their hosts through production of metabolites as a form of defence mechanism against predation (Huitu *et al.*, 2014) and pathogenic infection (Gomez-Lama Cabanas *et al.*, 2014). In return, the host provides protection, nutrients and a means of propagation via vertical-transmission (Faeth & Fagan 2002). Endophytes confer positive effects to their host, such as increased growth and development, and assistance in adaptation to environmental stressors (Faeth & Fagan 2002). Endophytes are known to produce several bioactive compounds that are either novel or those specific to the host. Numerous host specific compounds have been produced by endophytes that reside inside plants. Prominent examples of such compounds would be Paclitaxel, Vincristine, Vinblastine, Camptothecin and its derivatives (Kumar *et al.*, 2013; Palem *et al.*, 2016; Kuria Kose *et al.*, 2016; Chakravarthi *et al.*, 2008).

Fungi are an essential part of the ecosystem’s cycle of decomposition and recycling of organic materials, such as those of mycorrhizal fungi that colonizes plant roots and fungal endophytes in plant tissue (Rodriguez *et al.*, 2009). The importance of endophytic fungi lies in their contribution to major fields in industry as bio-control and increased plants productivity (Wakelin *et al.*, 2007), such as *Pestalotiopsis* sp. producing phyto-hormone indole-3-acetic acid (IAA) which stimulates development (Hoffman *et al.*, 2013).

Endophytic fungi are also a valuable source of drug candidates in pharmaceuticals as producers of antibiotics and anticancer compounds (Cui, Guo & Xiao 2011; Jouda *et al.*, 2016), antiviral (Wellensiek *et al.*, 2013) and anti-inflammatory agent (Stierle & Stierle 2000).

Investigation into the production of compounds in endophytes suggests that horizontal gene transfer is possible from the host to endophytes, an important factor in genome evolution (Jia *et al.*, 2016; Tan & Zou 2001). Anticancer drug Paclitaxel (Taxol) isolated from Taxus brevifolia was also found in endophytic fungi *Taxomyces andreanne*, demonstrating that endophytic fungi are able to produce compounds are a source of valuable compounds, due to the distribution of biosynthetic genes across host and endophytes (Yang *et al.*, 2014). This ability of endophytic fungi in producing valuable compounds also allows for the prevention of treasured plant diversity from bio-prospecting.
One of the outstanding aspect of marine research is the diversity of organisms where in a study comparing marine and terrestrial biodiversity showed that the marine ecosystem contains a much greater dimension in species distribution, pointing to a wider range in phyletic diversity (Carr *et al.*, 2003). It is hypothesized that there is a link between the arrays of biodiversity and the chemical diversity of marine natural products (Hay & Fenical 1996). Fungi metabolites possess unique structural compositions, unusual in drug research, as a result of the lack of research on the marine-derived endophytic fungi associated with marine invertebrates. Nonetheless, there are work on terrestrial isolated fungi and on metabolomics- the production of metabolites (Keller, Turner & Bennett 2005), and the study and manipulation of fungal gene clusters, which are capable of generating a multitude of bioactive secondary metabolites (Bok *et al.*, 2006).

In natural product research, biosynthesis of compounds is unpredictable but the realization of novel compounds is possible under laboratory condition through the manipulation of variables such as nutrient source, abiotic factors of temperature, pH, salinity, and even through the introduction of biotic stressors. While under controlled culture conditions some biosynthetic genes are phenotypically silent but can be induced through methods such as one strain many compounds OSMAC. This technique however is only viable if the strain is inherently capable of producing the particular metabolite, otherwise cultivation, even under correct conditions would not result in generation of the desired secondary metabolite. Other methods of metabolite induction are treatment with epigenetic modifiers (Cichewicz 2010) or microbial competition to activate secondary metabolite production (Netzker *et al.*, 2015).

In fungi it is found that the diversity of its genome is markedly different, despite some shared similarities in morphology (Galagan *et al.,* 2005). Studies of the fungal genome have provided clues as to their biosynthetic potential and activation of silent gene clusters through microbial introduction (Brakhage & Schroeckh 2011). One direction of drug discovery is marine-derived endophytes and their production of bioactive metabolites, as a treasure trove of diverse compounds with unique halogenated functional groups (Cabrita, Vale & Rauter 2010; Pauletti *et al.,* 2010; Zhang, Li & Wang 2016), bearing examples of novel indole alkaloids and phenolic sesquiterpenoids isolated from marine-derived Aspergillus species (Hasan *et al.,* 2015). Fungal genomic gene clusters have innumerable promise in generating secondary metabolites under certain conditions (Li *et al.,* 2016), such as the discovery of new compounds from fungus Asperigllus terreus from the marine sediments in response to changes to salinity level (Wang *et al.,* 2011), and changes in secondary metabolite production according to osmotic and salinity stress (Overy *et al.,* 2017).

The total scale of marine fungi species is most probably greatly underrated. marine fungi are associated to several organisms, most importantly invertebrates, algae and plants. While in the case of invertebrates (e.g. corals, ascidians, holothurians, gorgonians and sponges) and algae, fungi usually interact by association, when concerning marine plants, marine fungi habitually act as endophytes (Debbab *et al.*, 2012). An endophyte is a fungal or bacterial microorganism that colonizes a plant or algae either at an intra-cellular or inter-cellular level, this colonization takes place without an apparent pathogenicity towards the host and may occur during a part or the whole of the host's life cycle (Tan & Zou, 2001). In the case of endophyte-host plant interaction, fungi secondary metabolites have been proven to contribute to the host's survival and performance, by affecting factors such as chemical defense against predation, competitors and pathogens, growth rate, salt tolerance, photosynthesis and overall fitness, amongst others (Debbab *et al.,* 2012).

Fungi have been proposed as being able to improve the stability of the host skeleton, enable chemical defense against predators and competitors, parasitize pathogens, enhance nutrition and stimulate the host's immune system (Selvin *et al.,* 2010). This intricate relationship provided by association or endophytic behavior proves itself interesting for bioactive compound research since the colonization process and fungal-host interaction relies on chemical communication.

The grand entry of taxol, the world's first billion dollar anticancer drug, had augmented the importance of endophytes and shifted natural product research to endophytic fungi. The discovery of anticancer drug taxol was from the yew tree *Taxus brevifolia* in the 70s. The view of Stierle et al. was that yew trees might support certain endophytic microorganisms that may also synthesize taxol. Fortunately, Strobel et al. identified a taxol producing endophytic fungus, *Taxomyces andreanae*, from *T. brevifolia*. Screening for endophytes from other species of yew tree has highlighted another taxol-producing fungus, *Pestalotiopsis microspora*, from *Taxus wallachiana* .Therefore, the presence of a microbial source for a valued product could eliminate the need to harvest slow-growing rare yew trees, and the reduced cost makes it available to the world population. Secondary metabolites have long been produced as an adaptation for specific functions in nature. They offer a vital role in vivo; metabolic interactions between fungi and their plant hosts (i.e., signaling and defense and regulation of the symbiosis). A combinatorial approach using different defense strategies and the integration of a variety of metabolites enables the algae to survive in a highly competitive environment. Based on present literature survey, brown algae contributed the maximum followed by red and g reen algae. The comparatively short life cycle of Chlorophyceae as well as slow growth of endosymbionts together might have accounted for the low fungal diversity and bioactivity in green algae . Hundreds of natural products, including alkaloids, terpenoids, flavonoids, steroids, phenols, and quinines, have been obtained from algicolous endophytes.

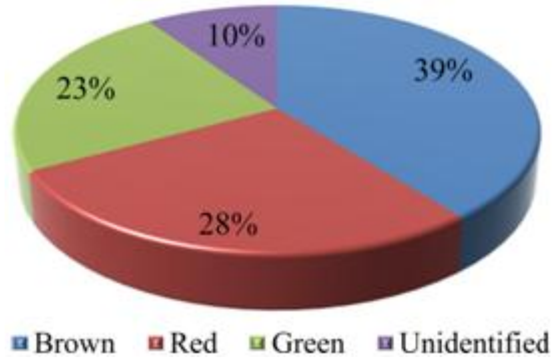

**Figure 3:** Proportion of bioactive compounds from different macroalgae (Source: Marine Algicolous Endophytic Fungi - A Promising Drug Resource ofthe Era, Manomi Sarasan)

Combining the particulars of the marine environment and microbial versatility, marine microorganisms have been considered as a new frontier for finding novel pharmaceutical candidates. According to the "one strain, many compounds" concept, different culture conditions result in the chemical diversity of the compounds. Therefore, various fermentation conditions, (i.e., shaking versus static cultures, application of different temperatures, light, aeration regimes, etc.) have been exercised. Fermentation media (viz., potato dextrose agar, barley spelt solid substrate, liquid Wickerham's medium, or malt extract medium) have been used for the successful scale-up of the production of bioactive compounds from endophytic fungi. Depending on fungal growth, cultures require an incubation period of 2-4 weeks after inoculation. The separation of mycelia and filtrate followed by the extraction of compounds using suitable organic solvents (viz., ethyl acetate, methanol, $CH_2Cl_2$, $CHCl_3$, and hexane) are performed. Indeed, ethyl acetate appears to be the most commonly used solvent in the bioactive screening of fungal endophytes. The cell-free organic phase must be concentrated to dryness under vacuum at 35-40°C using a rotary evaporator. Extracts are fractionated by column chromatography with polarity-based gradients of an appropriate solvent system, and the presence of compounds is detected by thin-layer chromatography. Further purification is done by reverse phase high-performance liquid chromatography followed by liquid chromatography mass spectrometry for identification of the compound by correlating both molecular weight and UV absorption data. Structural elucidation is done with the aid of high-resolution nuclear magnetic resonance and Fourier transform ion cyclotron resonance mass spectrometre.

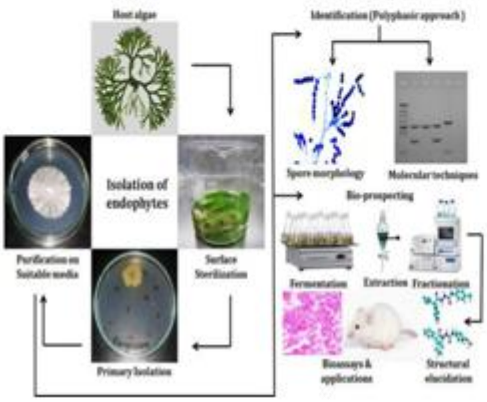


**Figure 4:** Bioprospecting of algicolous endophytes. (source: Marine Algicolous Endophytic Fungi - A Promising Drug Resource of the Era, Manomi Sarasan)

## 2. Conclusion

An increasing number of marine natural products are of interest as potential drugs. Apart from chemical and ecological reasons, it is thus of major importance for the evaluation of the pharmacological potential of marine natural products in order to confirm their efficacy as the best producers of beneficial compounds, possibly followed by cloning of the biosynthetic genes and biotechnological production. Despite of the fact that a wide range of antibacterial, antifungal, anticancerous and other biologically active agents have been found to be clinically significant, the development of marine microbes derived antibiotics is still in its juvenile stage. In conclusion, this study is identification of natural products capable of cytotoxic activity in cancer cells and antibacterial activities have become an important goal of research in bio-active products.

## References

[1] Abarca-Vargas, R, Pena Malacara, CF & Petricevich, VL 2016, 'Characterization of Chemical Compounds with Antioxidant and Cytotoxic Activities in Bougainvillea x buttiana Holttum and Standl, (var. Rose) Extracts', *Antioxidants (Basel), vol. 5, no*. 4, Dec 02

[2] Adeosun, CB, Olaseinde, S, Opeifa, AO & Atolani, O 2013, 'Essential oil from the stem bark of Cordia sebestena scavenges free radicals', *Journal of Acute Medicine*, vol. 3, no. 4, 2013/12/01/, pp. 138-141.

[3] Adham, M, Kurniawan, AN, Muhtadi, AI, Roezin, A, Hermani, B, Gondhowiardjo, S, Tan, IB & Middeldorp, JM 2012, 'Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation', Chin J Cancer, vol. 31, no. 4, Apr, pp. 185-96.

[4] Ahamed, A & Ahring, B 2011, Production of hydrocarbon compounds by endophytic fungi Gliocladium species grown on cellulose.

[5] Akl, MR, Ayoub, NM, Ebrahim, HY, Mohyeldin, MM, Orabi, KY, Foudah, AI & El Sayed, KA 2015, 'Araguspongine C Induces Autophagic Death in Breast Cancer Cells through Suppression of c-Met and HER2 Receptor Tyrosine Kinase Signaling', *Marine Drugs, vol*. 13, no. 1, pp. 288-311.

[6] Alagammal, M, P, DT & Mohan, V 2012, GC-MS analysis of Polygala rosmarinifolia Wights & Arn.

[7] Alfano, CM, Imayama, I, Neuhouser, ML, Kiecolt-Glaser, JK, Smith, AW, Meeske, K, McTiernan, A, Bernstein, L, Baumgartner, KB, Ulrich, CM & Ballard-Barbash, R 2012, 'Fatigue, inflammation, and omega-3 and omega-6 fatty acid intake among breast cancer survivors', J Clin Oncol, vol. 30, no. 12, Apr 20, pp. 1280-7.

[8] Alfarouk, KO, Stock, C-M, Taylor, S, Walsh, M, Muddathir, AK, Verduzco, D, Bashir, AHH, Mohammed, OY, Elhassan, GO, Harguindey, S, Reshkin, SJ, Ibrahim, ME & Rauch, C 2015, 'Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp', *Cancer Cell International, vol.* 15, no. 1, July 15, p. 71.

## Author Profile

**Dipika Madhukar Magdum** received the B.Sc. and persuing M.Sc. degree in Biotechnology from REVA University, Bengaluru in 2018 and 2020, respectively. She is working on research project in Indian Institute of Science, Bengaluru