REVIEW

Extraction and Preservation Protocol of Anti-Cancer Agents from Marine World

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Extraction and Preservation Protocol of Anti-Cancer Agents from Marine World

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Abstract
Marine source plays an important role in the form of pharmaceutical care and for the discovery of new molecular structures (i.e. targets). Marine organisms are a source of new therapeutics, especially for oncology, as a tremendous chemical diversity is found in marine bacteria, fungus, cyanobacteria, seaweeds, mangroves, microalgae and other halophytes. Several marine-derived compounds are currently extracted and synthesized by chemical processes for cancer treatment. By studying various papers related to marine source for new therapeutics for cancer treatment instead of other chemical enriched sources, the marine sources are largely unexplored for anti-cancer lead compounds. Hence this paper reviews results on the aspect with a view to provide basic information about the methods to produce extracts from marine organisms that are unique and different from that used by marine natural products chemists previously, yields both organic solvents and water soluble material for anti-cancer screening purpose. Chemists synthesize these compounds and their analogues in the laboratory for studying their activity towards various cancer cell lines.

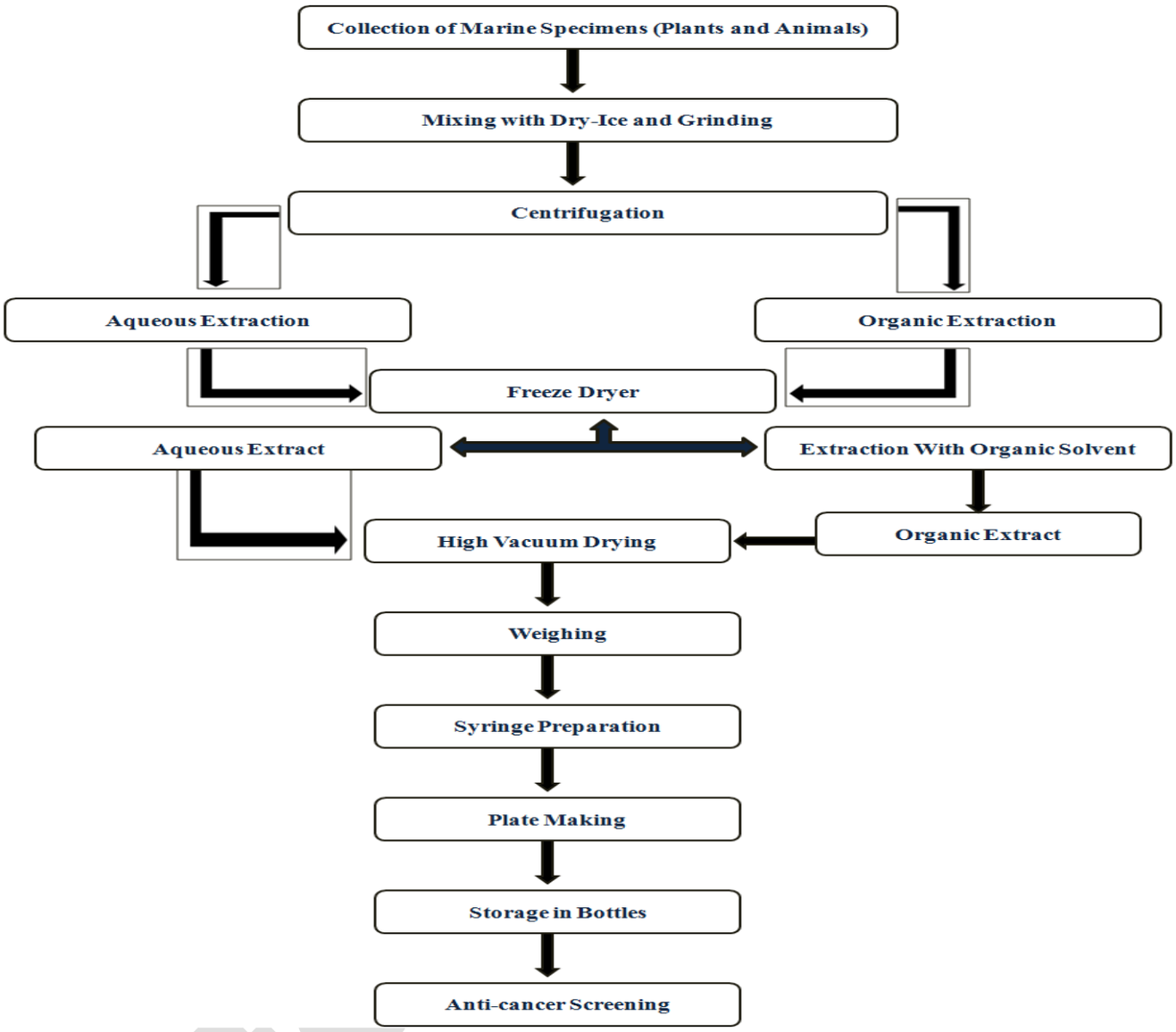
Keywords: Marine anti-cancer agents; marine microbes; fungi; algae; bacteria; cyanobacteria; anti-cancer screening.

1. Introduction

Cancer is a class of diseases in which a cell, or a group of cells represents uncontrolled growth (i.e. division beyond the normal limits), invasion (i.e. intrusion on and distortion of adjacent tissues), and metastasis (spread from one part to another part in the body through lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize while malignant tumors are not self-limited and metastasize. Most cancers form a tumor. The oncology is the branch of medicine that deals with the study, diagnosis, treatment, and prevention of cancer. Cancer is a human tragedy that affects people at all ages with the risk for most types increasing with age. It caused about 13% of all human deaths in 2007 (7.6 million). Cancers are primarily an environmental disease with 90–95% of cases due to modification in lifestyle and environmental factors and 5–10% due to genetics. Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutation that occurs from metabolism). Common environmental factors leading to cancer death include: tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation, stress, lack of physical activity, and environmental pollutants. These environmental factors cause abnormalities in the genetic material of cells [1]. For the treatment of cancer, limited number of effective anti-cancer drugs are currently in use, even though they have higher cases of nausea, vomiting, diarrhea, skin rashes, and headache, etc. so that there is real need for new, side-effect safe, cheap, and effective anti-cancer drugs to combat this dreaded disease. Natural products continue to be a major source of pharmaceuticals and for the discovery of new molecular structures [2]. The efforts to extract drugs from the sea started in the late 1960s. However, the systematic investigation began in the mid-1970s. During the decade from 1977 to 1987, about 2500 new metabolites were reported from a variety of marine organisms. These studies have clearly demonstrated that the marine environment is an excellent source of novel chemicals, not found in terrestrial sources.

So far, more than 10,000 compounds have been isolated from marine organisms with hundreds of new compounds still being discovered every year. About 300 patents on bioactive marine natural products were issued between 1969 and 1999 [3]. Some marine organisms proved to be the potent sources of drugs. These are mostly marine invertebrate animals like sponges, soft corals, sea fans, sea hares, nudibranchs, bryozoans, tunicates, etc. Some of the compounds derived from marine organisms have antioxidant properties and anti-cancer activities, but they are largely unexplored. Marine products have been used for medicinal purposes in India, China, the Near East and Europe, since ancient times [4]. Some speedy expansions in molecular biology, bioengineering, genomics, proteomics, and metabolomics have aided the discovery of natural products and their implication in anti-tumor drug development. Approximately, 15,000 marine specimens in > 1600 genera and > 6100 species of marine animals, >450 genera and >1200 species of marine plants have been processed.

Figure 1: Flow scheme for the production of anti-cancer compounds from marine organism by extraction process.



2. Extraction Protocol

The object of this protocol is the discovery of new anti-cancer agents from marine plants and animals. To achieve this object from both marine plants and animals, compounds must be extracted, concentrated, and preserved during storage without any change in the chemical composition during these processes. Then this extract is used for the detection of anti-cancer activity among different cell lines. Here complete marine extraction processing method utilized by NCI-Fredrick has been demonstrated [5].

2.1. Collection of specimens

Marine specimens, both animals and plants, having anti-cancer activity, were obtained through competitive contracts with those having expertise in the flora of various regions of the world. All marine specimens were purchased with the provision given in a NCI Letter of Collection; most of them have been obtained from the general region of the South Pacific and collected by scuba diving. One of the most important properties of marine specimens is fast degradation; therefore, these samples are quickly frozen with dry ice and stored frozen at −20°C until they are processed. In determining anti-cancer activity, more than one extracts from different parts of single marine animal or plant show a “positive” anti-cancer activity.

2.2. Grinding procedure

For the extraction process, grinding was a primary process for the new Anti-cancer Drug Discovery Program that was established in 1987, as the extracts which were produced were to be used as the "feed-stock" for different new human cancer cell line panel which was to serve as the primary discovery tool used for the detection of anti-cancer lead compounds [6, 7]. Marine frozen tissue pieces and dry ice pellets are slowly added into the grinder. The amount and speed of sample addition is dependent upon the specimen's characteristics and only experienced operator will assure infrequent jams. It will jam on occasion and it is imperative that the machine be turned off immediately to avoid over loading the circuitry and or damaging the gear box of the machine. Approximately, double the volume of dry ice is added to the specimen to keep the mechanism and housing cold during grinding. There are few marine animals such as starfish, sea squirts, holothurians etc. that remain gummy even at dry ice temperature and thus do not grind well. These are also broken into pieces when mixed with dry ice, but just prior to grinding, liquid nitrogen is poured over the combined dry ice pellets and tissue pieces. At liquid nitrogen temperature, every marine specimen can be ground. Pieces of specimen tissue are "chased" from the throat by adding more dry ice, and then the throat is taken apart and any remaining specimen was added to the ground material in the bag. At this point, the contents of the bag include the finely ground tissue of the marine specimen including shell if present, the ground saltwater ice which was a part of the specimen and powdered dry ice. A tag with appropriate identifying barcode label is attached to the bag with wire, and twisted securely. The bag containing this ground sample is placed inside at −20ºC freezer, where it will stay for generally 3 days, during which time the dry ice sublimes. At completion of grinding of each specimen, the head is removed from the body of the machine, disassembled, cleaned thoroughly by placing it in the sink, and immediately dried to avoid rust formation. When reassembling, all possible sticking points are sprayed with cooking corn oil or similar food grade lubricant. Petroleum oil is not used for lubrication because it would be toxic to cells in tissue culture and should never be allowed to contaminate an extract. To avoid cross-contamination and hazards to technicians, the work area is thoroughly cleaned, with any potentially contaminated materials, plastic bags, boxes, gloves, dust masks, etc. Disposed and subsequently incinerated. Marine invertebrates and wet-frozen marine plants, wet-frozen higher plants from a saline environment, wet-frozen fungal basidiocarps, and waxy nuts/fruits have all been finely ground successfully by this protocol. Hard corals, bivalves, and other animals with hard shells are crushed with a hydraulic press prior to grinding, but the shell is passed through the Hobart grinder as well. A number of other grinding techniques were tried during the methods development study, with the Hamburger grinder of the type mentioned above found to be most suitable for this grinding while frozen protocol.

2.3. Extraction processes

For the extraction of anti-cancer agents from the above ground marine specimen, two extracts are to be made. (1) Aqueous extraction is done by centrifugation. (2) The drymarc is re-extracted by the organic solvent mixture DCM/MeOH (1:1).

2.3.1. Aqueous extraction by centrifugation

Aqueous extraction of a ground marine specimen takes place 3 to 4 days following the grinding and after all the dry ice has sublimed from the specimen. The bag containing the ground specimen is removed from the freezer, its contents are transferred into a 4 L beaker and sufficient milli-Q high purity water is added to make an about 3:1 mixture. This is the first time the marine specimen has not been frozen since its collection. Mixing is done while inside a refrigerator at about 4ºC, through the use of a mechanical device such as the ConTorque motorized paddle stirrer for about 45 minutes until homogenous, ice-free aqueous slurry has been achieved. The centrifuge rotor is prepared by lining the rotor with a strip of pre-cut Whatman 3 mm chromatography paper. With the paper set firmly against the walls of the rotor and moistened with high purity water, it will adhere to the rotor wall with no bubbles or creases. With a 4 L flask in wet ice set to receive the filtrate coming through the draining hose from the rotor catch-basin and with the rotor spinning at 1200 to 1500 rpm, the aqueous slurry is slowly added to the center of the basket rotor. The rotor speed may be increased to hasten filtering, but speeds above 2000 rpm are rarely required and may compress the filter cake thus slowing the flow rate. Set rotor speed above 2500 rpm with this type of rotor. Aqueous extracts, which are often 3 to 5 L for a 1 kg specimen, are poured into barcode-labeled stainless steel trays suitable for freezing and freeze-drying. The filter paper with compressed tissue marc attached is removed from the rotor and placed in a properly labeled dish. The aqueous-soluble extract and marc plus paper are frozen in -40ºC "sharp" freezers, and then lyophilized. Freeze-drying of marine aqueous extracts and marcs typically takes 5 to 7 days when the ice cake is 3 cm thick. Following freeze-drying and transfer into borosilicate bottles, the aqueous extract is weighed and an aliquot taken for screening. The drymarc is also weighed, and then re-extracted with DCM/MeOH to produce the organic solvent extract for screening. Various problems may occur during centrifuging, such as torn paper, clogged paper, and marc material into the receiving flask. Torn paper and marc are removed and placed into a labeled dish, then a new filter paper is placed in the rotor and centrifugation is started again, passing the aqueous fraction which has been collected through the filter paper a second time. Scraping a clogged paper is often an effective way to restore the filtrate flow, but care must be taken not to tear the filter paper in doing so. With experience, one may be able to foresee a clogging problem from the consistency of the slurry (i.e. a difficult holothurian) and therefore try to forestall the problem with the use of a filter aid (i.e. Celite). Placing a coarse pre-filter of fiber glass on top of the paper filter inside the basket rotor allows

for filtration of some difficult, slimy materials. Occasionally nothing works, and an entire sample is frozen and lyophilized. In any case, all extra weights, i.e. paper, fiber glass, Celite, etc., which have been added to the marc, and will remain after lyophilization, are recorded. These aqueous extracts contain salt, and that saltwater is very corrosive to metal parts, so thorough and frequent washing of apparatus is important. The basket rotor should be cleaned, taking care to remove residue from underneath the rim, and then dried carefully. The inside of the centrifuge is rinsed and wiped dry after each specimen has been done. Glass wares are washed thoroughly and promptly after emptying. All items are rinsed well with high purity water before being put away.

2.3.2. Drymarc is re-extracted by organic solvent

Lyophilized marine marcs are setup for extraction with organic solvent immediately upon removal from the freeze drier, or if that is not possible, then stored under vacuum. Marcs that contain spicules can be extreme skin irritants in much the same way as coarse fiber glass and spicules are not the potential irritants. During handling, these marine marcs wearing thick rubber gloves and along sleeved lab coat with cuffs secured and inside a hood, is standard procedure in this extraction lab and is strongly recommended to others who may be engaged in this process. Although the wet-frozen weight of each specimen had already been recorded in earlier step, in order to calculate the potential yield of obtained from one of these extracts, having the dry weights of both aqueous extract and marc is required. Therefore, the lyophilized marc in its vessel is placed on a balance and the gross weight is measured. Then the marc along with the filter paper is transferred into a percolator. The empty vessel is returned to the balance and its weight subtracted. The weight of the filter papers and/or filter aids is also subtracted from the gross weight to obtain the lyophilized weight of the marine tissue itself, which is recorded in the database. If this weight and the weight of the lyophilized aqueous extract are added, an approximation of the weight that would have been obtained had the entire, original wet-frozen specimen been lyophilized can be calculated. From this, an approximate yield of a molecule of interest from either water extract or organic extract can be calculated, and an estimate of the yield per kg from the original collection may be obtained. Inside the percolator, any sizable clumps of marc are broken into small pieces, and pressed down to minimize the volume of solvent required. Enough 1:1 DCM/MeOH is added to cover the marc with at least one inch of solvent. Marine marcs tend not to swell in organic solvent. After soaking overnight, the organic solvent is drained from the percolator and the marc is covered again this time with pure MeOH. After soaking another half-hour, the MeOH has been drained and the organic solvent extracts are evaporated to give a concentrate extract. Organic solvent extracts of marine marcs dry quickly without foaming. The concentrate is transferred into an appropriate storage bottles or a vessel and further dried by applying a high vacuum dryer, weighed and the weight of this dry extract is recorded.

2.4. Storage and preparation of plate

At the end of 2009 more than 29,800 marine invertebrate extracts, 4200 marine plant extracts had been produced by the methods given above. From each 120 mL borosilicate glass “bulk bottle” an aliquot is weighed for the production of micro titer plates. Aliquots in plastic tubes, each individually bar-coded, are solubilized into an appropriate solvent, which is transferred by a TECAN robotic liquid handling system into multiple polypropylene 96 well, deep-well micro titer “Master Blocks.” When solvent is removed, each well contains a dry weight of 15 mg, which is an amount sufficient for hundreds of test plates to be produced for screening. Each of these “Master Blocks” is high vacuum dried and a heat-seal laminate applied prior to storage or shipment. At shipment, all data generated in the National Plant Safety Goals (NPSG) is uploaded to the Natural Products Repository Support System database. Extracts in borosilicate bottles, along with more than 1,70,000 micro titer plates containing aliquots of 88 different extracts per plate, reside in −20ºC freezers at the NCI-Frederick Natural Products Repository in Frederick, MD, available for call-up. At the end of the extractions and still growing, this must certainly be among the largest and most diverse libraries of natural products crude extracts assembled for drug discovery anywhere in the world. Yet, as large as it is, only ~10% of the known higher plant species are present, and lesser percentages of marine organisms and fungi. This library is considered a ”National Resource,” available in both 96- and 384-well micro titer plate formats, ready for use not only by NCI lab sat Frederick but elsewhere. Thus, the entire library can be screened on about 2600 unique 96-well micro titer plates. In addition to the initial screening in the DTP60 human cell anti-cancer assay at NCI-Frederick, this library has been utilized in a number of diverse bioassays, such as anti-microbial, anti-HIV, and various whole-cell and molecular target assays.

2.5. Marine-derived anti-cancer agents

Marine source are rich source of natural products and many compounds are derived from these sources. The anti-cancer drugs that have been isolated from marine organisms such as bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes, etc. have been shown to possess cytotoxic activity against various tumors.

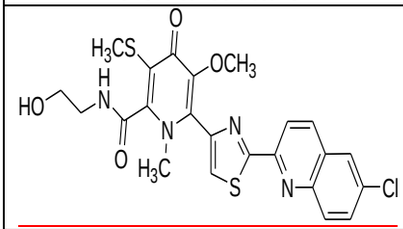
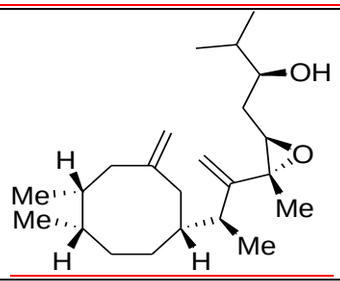
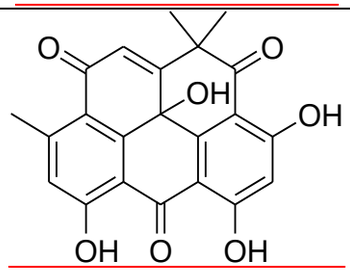
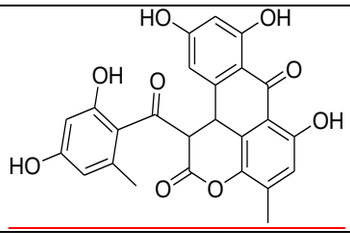
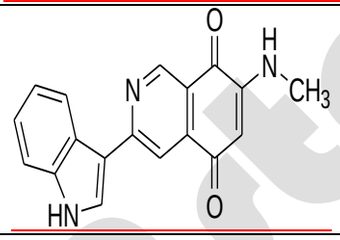
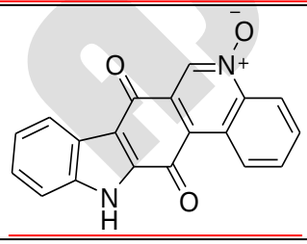
Table 1: Marine-derived anti-cancer agents, chemical classification, mechanism of action and sources [8].

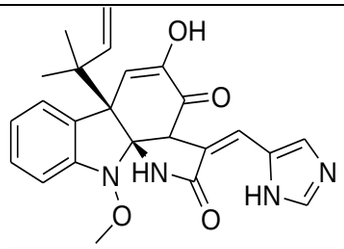
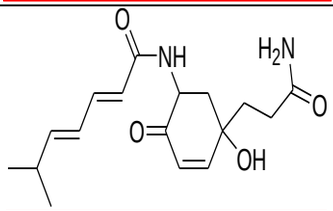
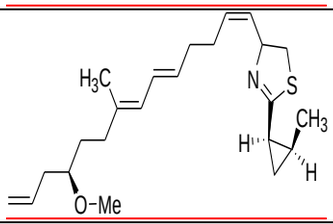
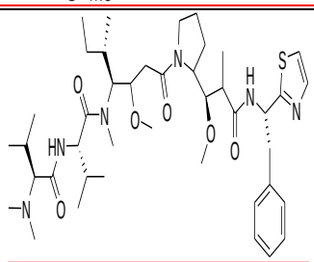
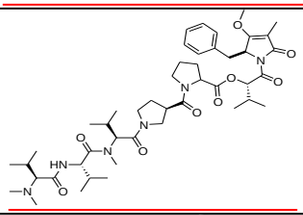
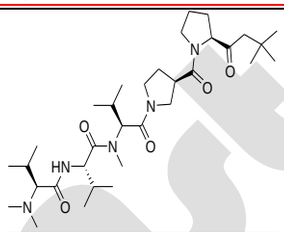
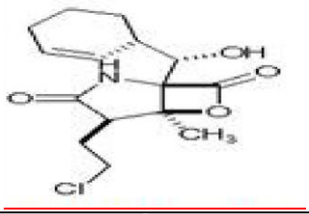
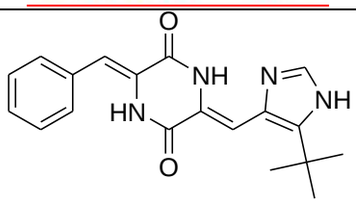
S. No.	Compound	Chemical class	Mechanism of action	Sources
1a.	Citarabin	Nucleoside	Inhibit DNA polymerization	Sponge
1b.	Eribulin mesylate (E7389)	Macrolide	Interfere with microtubulin	Sponge
1c.	Sorbicillactone-A	β -Lactone -γ-lactam	Anti-leukemic agent	Sponge
1d.	HT1286	Dipeptide	Interfere with microtubule	Sponge
1e.	LAF389	Amino acid derivative	Methionine aminopeptididase inhibitor	Sponge
1f.	Hemiasterlin (E7974)	Tripeptide	Interfere with microtubulin	Sponge
1g.	KRN 7000	A-galactosylceramide	Immunostimulatory	Sponge
1h.	Halicondrin B	-	-	Sponge
1i.	Discodermolide	Polyketide	Stabilization with tubulin	Sponge
2a.	Plitidepsin	Depsipeptide	Apoptosis inducer	Tunicate
2b.	Trabectedin or Ecteinascidin (ET-743)	Alkaloid	Treatment of soft tissue sarcoms	Tunicate
2c.	Didemnin B	-	-	Tunicate
3a.	Isogranulation	-	-	Brazilian tunicate
4a.	Aplidine	Depsipeptide	Apoptosis inducer in vitro and vivo	*Aplidium albicans* tunicate
5a.	Soblidotin (TZT 1027)	Peptide	Interfere with microtubulin and vascular disrupting agent	Bacterium
5b.	Tasidotin, Synthadotin (ILX-651)	Peptide	Interfere with microtubulin	Bacterium
6a.	LY355703, CRYPTO 52	Cryptophycin	Interfere with microtubulin	Cyanobacteria
6b.	Depsipeptide (NSC 630176)	Bicyclic peptide	Inhibit histone deacetylation	Cyanobacteria
7a.	MarizomibSalinosporamid-A (NPI-0052)	Lactam	Inhibit proteasome	Bacteria
8a.	Bryostatin 1	Polyketide	Inhibit PKC isozyme	Bryozoan
9a.	Dolastatin 10	-	Inhibition of microtubules and proapotic effects	Sea hare
10a.	Plinabulin (NPI-2358)	Diketopiperazine	Interfere with tubulin and vascular disrupting agent	Algae
11a.	Squalamine lactate	Aminosterioid	Calcium binding protein antagonist	Shark
12a.	Elisidepsin	Depsipeptide	-	Mollusc
13a.	Zalypsis	Alkaloid	Cell cycle arrest	Nudibranch
14a.	6-Hydroxymethyl acyl fulvene	Omphalotusolearius	-	Mushroom

3. Conclusion

With the help of these extraction and preservation protocols of marine specimens it was found that these marine organisms have biologically active, chemically sensitive molecules for anti-cancer screening purpose. These anti-cancer compounds are already discovered in the library of extracts to tests the preservation of the bioactive molecules originally present in marine specimens by the processing methods. Moreover, the traditional organic solvent-soluble marine specimen portion has been used from which most traditionally and presently used, small molecule drugs have been obtained. In addition to the diversity of new, anti-cancer compounds, active substances have now been shown to be present in water extracts. Thus, unknown number of bioactive molecules will be reused many times as the feed-stock for the determination of new anti-cancer activity as they are developed. The new molecular targets are discovered and as a challenge for chromatographers, natural products chemists, and chemists for synthesizing these compounds and their analogues for anti-cancer screening in future years.

Table 2: Marine-derived anti-cancer agents with chemical structures.

Chemical Structure	Name	Use in cancer	Sources	Reference
	Lodopyridone	Cytotoxic to HCT-116 human colon cancer	Marine sediment *Saccharomonospora* sp.	[9]
	Octalactin A	Cytotoxic to murine leukemia L1210-Leukemia cells	Marine bacteria *Streptomyces* Sp.	[10]
	Resistoflavine	Potent cytotoxic activity against cell lines Gastric adenocarcinoma and hepatic carcinoma in vitro	*Actinomycetes*	[11]
	Aspergiolide A	Cytotoxicities against A-549, HL-60, BEL-7402, and P388 cell lines	Marine fungus *Aspergillus glaucus*	[12]
	Mansouramycin D	Cytotoxicity towards 36 tumor cell lines and selectivity for non-small cell lung cancer, breast cancer, melanoma, and prostate cancer cells	Marine-derived *Streptomyces* sp.	[13]
	Calothrixin A	Inhibit the growth of human HeLa cancer cells	Marine algae	[14]

	Melegrin	Anti-tumor	Marine fungi	[15]
	Daryamide C	Cytotoxic activity against the human colon carcinoma cell line HCT-116	*Actinomycetes*	[16]
	Curacin A	Anti-microtubule	Cyanobacteria *Lyngbya majuscula*	[17]
	Dolastatin 10	Anti-microtubule	Cyanobacteria	[17]
	Dolastatin 15	Anti-microtubule	Cyanobacteria	[17]
	Tasidotin	Cytotoxic towards Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma, and synovial sarcoma lines	Cyanobacteria	[18]
	Salinosporamide A	Potent in vitro cytotoxic activity against many tumor cell lines	Marine bacterium *Salinispora tropica*	[18]
	Plinabulin	Anti-cancer agent and Vascular disrupting agent	Marine bacteria	[18]

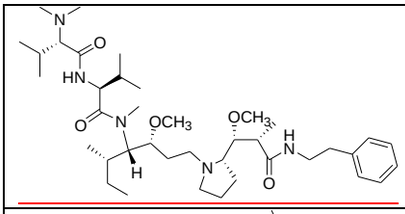
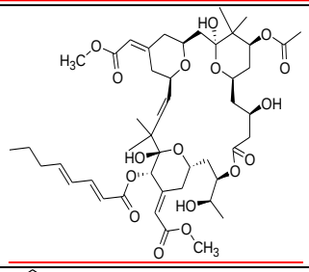
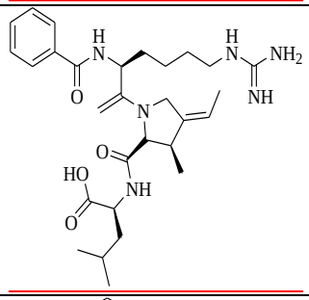
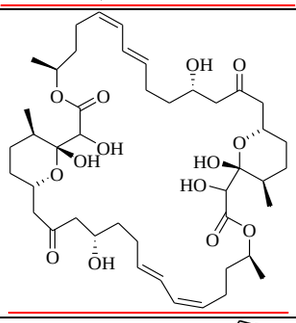
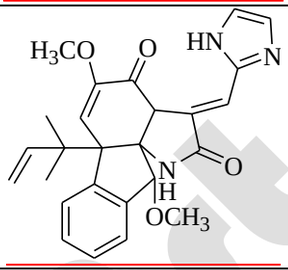
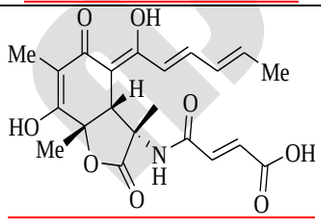
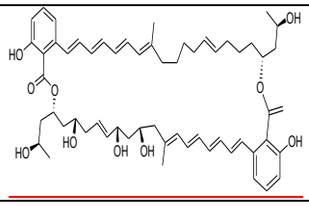
	Soblidotin	Microtubule-depolymerizing agent exerts both a direct cytotoxic activity against cancer cells	Marine bacteria	[18]
	Bravostatin 1	HL-60 chronic lymphocytic leukemia, lung, prostate and non-Hodgkin's lymphoma tumor cells	Bryozoan (a sessile, moss-like marine animal) *Bugulaneritina*	[18]
	Lucentamycin	Significant in vitro cytotoxicity against HCT-116 human colon carcinoma	Fermentation broth of a marine-derived *actinomycete*	[19]
	Tartrolon	displayed strong cytotoxic activity against three human tumor cell lines	Marine-derived *actinomycetes*	[20]
	Oxaline	Anti-tumor oxaline disrupted cytoplasmic microtubule assembly in 3T3 cells. Furthermore, oxaline inhibited polymerization of microtubule protein and purified tubulin dose-dependently in vitro	Marine fungi	[21]
	Sorbiciloctone A	Exhibits a strong cytotoxic activity against L5178y leukemic cells, combined with a relatively low toxicity to cervical carcinoma HeLa S3cells and pheochromocytoma PC 12 cells	Isolated from the Mediterranean sponge *Ircinia fasciculata*	[22]
	Marinomycin A	Selective cancer cell cytotoxicities against six of the eight melanoma cell lines in the National Cancer Institute's 60 cell line panel	*Actinomycetes*	[23]

Table 3: Marine-derived anti-cancer compounds and their derivatives: family, biological activity and source.

Chemical	Family	Biological activity	Source	References
Microviridin, Toxin BE-4, Siatoxin	*Microcystisaeruginosa*	Anti-cancer	Microbial flora	[24, 25]
Daunorubicin	*Streptomyces peucetius*	Anti-cancer activities on acute myeloid leukemia and acute lymphocytic leukemia	Microbial flora	[26]
Borophycin	*Cynobactera Nostoc linckia and Nostoc spongiaforme* var. tenue	Cytotoxic against human epidermoid carcinoma (LoVo) and human colorectal adrenocarcinoma activity	Algal flora	[27]
Apratoxins	*Cynobacteria*	Inhibits a variety of cancer cell lines	Algal flora	[28]
Cryptophycin 1	*Nostoc linckia*	Cytoxicity against human tumor cell lines and human solid tumors	Algal flora	[29]
Cryptophycin 8	*Nastoc spongiaeforme*	Greater therapeutic efficiency and lower toxicity than cryptophycin 14 in vivo	Algal flora	[30]
Stypoldione	*Stypodium* sp.	Cytotoxic	Algal flora	[31]
Condramide A	*Chondria* sp	Cytotoxicity	Algal flora	[32]
Caulerphyne	*Caulerpa* sp	Cytotoxicity, anti-cancer, anti-tumor and anti-proliferrating activity	Algal flora	[33–35]
Meroterpenes and Usneoidone	*Cystophora* sp.	Anti-tumor	Algal flora	[36]
Largazole	*Symploca* sp	Anti-proliferative activity	Algal flora	[37]
Apratoxin A	*Lyngbya boulloni Leptolyngbya* sp.	Cytotoxicity to adrenocarcinoma	Algal flora	[28]
Coibamide A	*Leptolyngbya* sp.	Cytotoxic against NCIH460 lung and mouse neuro-2a cells	Algal flora	[38]
Scytonemin	*Stigonema* sp	Anti-proliferative and anti-inflamatory activities	Algal flora	[39]
Crude	*Acanthophora spicifera*	Tumoricidal activity on Ehrlich's ascites carcinoma cells developed in mice	Algal flora	[40, 41]
Crude	*Acanthophora spicifera*	Antioxidants and inhibiting cancer cell proliferation	Algal flora	[40, 41]
Phloroglucinol and its polymers eckol (trimer), Phlorofucofuroeckol A (pentamer), Dieckol and 8,8'-Bieckol (hexamers)	*Palmaria palmata*	Antioxidant activity of the phlorotannins	Algal flora	[42]
Phloroglucinol and its polymers eckol (trimer), Phlorofucofuroeckol A (pentamer), Dieckol and 8,8'-Bieckol (hexamers)	*Eisenia bicyclics*	Antioxidant activity of the phlorotannins	Algal flora	[43, 44]
Crude	*Sargassum thunbrergii*	Anti-tumor activity, Inhibition of tumor metastasis carcinoma cell	Algal flora	[45, 46]
Fucoidan	*Ascophyllum nodosum*	Anti-proliferative anti-tumor anti-cancer, anti-metastatics and fibrinolytic	Algal flora	[47, 48]
Lignins	*Ceriops decandra*	Antioxidant	Marine costal plants	[49]
Mangrove tea	*Acanthus illicifolius Ceriops decandra*	Anti-cancer	Marine costal plants	[50]
Ribose derivatives of benzoxazoline	*Acanthus illicifolius*	Anti-cancer	Marine costal plants	[51, 52]
Xanthone, Biflavonoids, Benzophenones, Neoflavanoids and Coumarin derivatives	*Calophyllum inophyllum*	Anti-cancer, anti-tumor and lipid peroxidation	Marine costal plants	[53, 54]
Diterpenes exhibited remarkable anti-tumor promoting activity in vivo on two-stage carcinogenesis test of tumor	*Excoecaria agallocha*	Anti-tumor activity ofmethanolic extract based on three assay (1) DPPH radical scavenging (2) Linolelic acid roxidation assay, and (3) Oxidative cell death assay	Marine costal plants	[55]

Authors' Contributions

All authors contributed equally to this work.

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