ISSN : 0974 - 7508 Volume 10 Issue 4

Natural Products

An Indian Journal

Full Paper

NPAIJ, 10(4), 2014 [102-112]

Chemistry and biology of sea cucumbers

Vijai Lakshmi[1,2*], Santosh Kumar Agarwal[2], Abbas Ali Mahdi[3], Ajet Saxena[1]

[1]Medicinal and process Chemistry division, Central drug research institute, Lucknow-226001, (INDIA)

[2]Phytochemistry division, Central institue of medicinal and aromatic plants, Lucknow-226015, (INDIA)

[3]Biochemistry department, King george medical university, Lucknow-226003, (INDIA)

E-mail : vijlakshmius@yahoo.com

ABSTRACT

The manuscript updates the chemical constituents isolated from various family of sea cucumbers biomass. A large number ofpure compounds of different classes have been covered. The manuscript described sources, bioactivities and chemical and physical constants of the different classes of compounds.

KEYWORDS

Sea cucumber species;
Chemical constituents;
Source;
Bioactivities.

INTRODUCTION

The phylum Echinodermata, which comprises about 6,000 living species distributed in

sea cucumbers are a fascinating group of marine invertebrates. They live chiefly among the corals but are also found among rocks and in muddy and sandy flats. They are distributed from the shore to the greatest depths. Their length ranges from a few millimeters to more than 2.0 meter and they occur in all color combinations white, black, red, blue, green, yellow, violet etc.[1]

Sea cucumbers are distributed over South Pacific region, Peru, Chile, Caribbean East Pacific (California, Mexico, Baja, Canada) West Atlantic, New Zealand, West Australia, Red sea, South East Asia and Indian Ocean. The species of highest commercial value in tropical waters of the western pacific and Indian Oceans are *Holothuria fuscogilva*, *Holothuria nobilis*, *Holothuria scabra*, and *Actinopyga mauritiana*, species of medium value include *Actinopyga echinites*, *Actinopyga miliaris* and *Thelenota ananas*. Species of low value include *Holothuria atra*, *Holothuria edulis*, *Bohadschia argus*, *Bohadschia vitiensis*, *Stichopus chloronotus* and *Stichopus variegates*. They are found in the Indian Ocean, Andaman & Nicobar Islands, in the Gulf of Mannar, Palk Bay and Lakshadweep.

The body of sea cucumber is elongated, leathery and muscular. Spines are contained within the skin. Sea cucumber is dioecious, but hard to differentiate between male from andfemale form.. Cuvierian tubules (defensive glands which extend from anus) contained a large amount of saponins, which are pesticidal.

TRADITIONAL USES OF SEA CUCUMBER

Sea cucumbers were recorded as a healthy life style tonic in the traditional medicine in many ancient writings. According to the Chinese traditional medicines, the sea cucumbers nourish the blood and vital essence, strengthen the kidney "qi" (treat disorders of the kidney and the reproductive organs) and moisten mucosal dryness (especially of the intestines). Other common uses including includes for treatment of weakness, impotency, debility of aged, constipation due to intestinal dryness and frequent urination. Traditionally sea cucumbers are eaten by Chinese people more for their

tonic value than for their sea food taste. Hence the popular Chinese name for sea cucumbers is "Hoisom", which mimics as the ginseng of the sea[2,3]. Holothurians and its products could cure certain ailments, hypertension and asthma, also heal internal wounds and in cancer[4]. Sea cucumbers also serve as a rich source of the polysaccharide chondroitin sulfate[5] which is well known for its ability to reduce arthritis pain by acting like a glucosamine sulfate. Sulfated polysaccharides also inhibit viruses; chondroitin sulfate have been potential for HIV therapy[5]. Chinese studies revealed that the sea cucumbers contained saponin glycosides[5] which have a structure similar to the active constituents of ginseng, ganoderma and other famous tonic Chinese herbs. Studies have also further indicated that anticancer properties are extracted from these sea cucumbers saponins and the polysaccharides. Vitamins such as B1, B2, B6, A, D and E are isolated in many species (*Thelonota ananas)*. Some trace elements, present within the body of these animals are Mn, Fe, Zn, Co, Cu and Se[5,6]. Certain extracts of the sea cucumbers had also been described to contain Vitamin E and antioxidants. Therefore sea cucumbers could provide a good source of external antioxidant[4] for human consumption. The toxins of sea cucumbers have antiviral, antitumoral, anticancerous and antifertility properties[1] thus can be exploited for production technology could be used in the pharmaceutical industries[1].

Russian, Japanese, and Chinese studies seem to support that these invertebrates contained saponins[5] with anti-inflammatory and anticancer properties[7].

In addition, the oil of the sea cucumbers contains two anti-inflammatory fractions[8]. One fraction has fatty acids characteristic of those found in fish; they can be used as a substitute for fish oil in reducing inflammation. The main compounds of interest in the fish oil are EPA (eicosapentaenoic acid also found in sea cucumbers and DHA (docosahexaenoic acid) which is unique to fish:

CHEMICAL CONSTITUENTS OF THE SEA CUCUMBERS

A large variety of the chemical compounds including saponins, sterols, sapogenins, ceramides, gangliosides and fatty acids have been isolated and reported by various research workers.

Saponins

Among the echinoderms, sea cucumbers and starfishes invariably contained saponins, which are generally responsible for their toxic nature[9]. Saponins, water-soluble compounds with complex structures. Saponins have been isolated from a large number of terrestrial plants, but are uncommon in animal constituents. In the animal kingdom they are almost ubiquitous in sea cucumbers and starfishes, while found rarely in others. Chemically, saponins from sea cucumbers are triterpenoid glycosides whereas those from starfishes are steroidal glycosides. In the past few years a large number of metabolites with cytotoxic, antifungal and antineoplastic activities have been isolated from sea cucumbers.

Glycoside fractions from 34 sea cucumber species were isolated by the precipitation with cholesterol and subjected to comparative examination. From glycoside fraction, hydrolysis products were investigated. A relation was established between the systematic position of animal and its glycoside content. The families Holothuriidae, Stichopodidae and Cucumariidae have different sets of glycosides[10].

In 1942, Yamanouchi first isolated a hemolytic and piscicidal oligoglycoside from the sea cucumber and named it as holothurin[11]. Afterwards, Matsuno and Iba and Hashimoto found that holothurin was also produced by another kind of sea cucumber *H. lubrica* and it comprised two triterpene-oligoglycosides designated as holothurin-A and holothurin-B. On the other hand, Nigrelli reported in 1952 the isolation of piscicidal and antitumor principle named holothurin from the sea cucumber *Actinopyga agassizi*[12]. The chemical structure of holothurin-A was characterizedby Chanley[13].

It is known that the cuverian glands of sea cucumbers are particularly rich in saponins. These are typically composed of carbohydrate and triterpenoid moieties, while asterosaponins are steroidal glycosides[14]. Sea cucumbers are known to contain a variety of triterpene glycosides of lanosterol type with a characteristic γ-lactone skeleton named holostane and a sugar chain containing up to six monosaccharide units. In addition, sulfate groups may be present at certain positions of the aglycon[15].

Saponins, which exhibit a wide spectrum of bio-

Full Paper

logical activities[16] are glycosides and are characterized by a number of common properties such as froth formation, haemolytic activity, toxicity effects to fishes and formed complex with cholesterol[17,18]. Per se various reviews dealing with the distribution, isolation techniques and characterization of triterpenoid saponins have been published[19,20]. Similarly, basic characters of steroidal saponins were the subject of two comprehensive reviews[21,22].

ISOLATION AND CHARACTERIZATION

Although the integration of silica gel column chromatography, semi preperative HPLC and repeated preparative TLC may yield expected separation of saponin in most cases. Dry column chromatography in conjunction with other chromatography techniques, such asHPLC and flash chromatography, have been used for separation. The technique of rotation locular counter current chromatography (RLCC) along with reversed phase chromatography and droplet counter current chromatography (DCCC) have been employed for the separation of saponins[23]. The isolation of some saponins necessitates sometimes pretreatment of material before subjecting them to the usual extraction and isolation procedures[24].

The Lieberman-Burchard test has been employed in most instances to detect saponins. It has also been used in differentiating the triterpenoidal and steroidal saponins, since the former give pink or purple color where as the latter exhibit blue-green colors. Thionyl chloride, phosphomolybdic acid, silico-tungstic acid, Molisch test, Rhodizonate test (sulfate group) and haemolysis of blood have generally been used for detection. Haemolytic activity was shown by Ginseng rodix saponin.

The characterization of a saponin is not an easy task and conventional methods include acid hydrolysis followed by characterization of the aglycones and sugar moieties. Earlier methods for structure determination of saponins are quite tedious and a great deal of chemical derivatization or degradation work. Some reactions like solvolysis and methylation followed by methanolysis are useful for characterization. These studies no doubt provide definite proof for the structure but at the same time, require the sufficient amount of compounds, which in most cases is not available. In addition, the major disadvantage of this method is the cyclization of furostanol to spirostanol aglycone and can yield several alternative structures to that of the original compound. A decision between various alternative structures cannot be achieved by FD-MS[25,26] and FAB-MS[27,28]. In this respect ^{13}C-NMR spectroscopy offers a convienient and non destructive method for studying the structure of a saponin as the sugar carbon resonances occurs largely in a definite region and they are quite distinct from the resonances of the sapogenin nucleus[29]. The newer techniques of mass spectroscopy particularly FD-MS and FAB-MS are of immense help in determining of molecular weights of the saponins and the sequence of their sugar units.

The saponins are composed of aglycon and sugar moieties, which are recovered after acid hydrolysis and are investigated separately. The method of photochemical degradation has been developed by Kitagawa et al for selective cleavage of the gluconoside linkage[30]. Smith degradation is sometimes employed for the determination of the sugar sequence in saponins[31]. Sapogenins are formed as a result of acidic or enzymatic hydrolysis of saponins or they occur as such in nature. Enzymatic hydrolysis has been frequently employed for specific cleavage of glycosidic linkages in the structure elucidation of saponins by using different enzymes, obtained from plants and animals (such as hesperidinase or snail enzyme) for the structure elucidation of holothurin-A&B.

All of the modern structural methods for the glycosides, NMR spectroscopy yields the most complete picture of saponin structure and behavior in solution with or without prior structural knowledge. A NMR spectrum (^{1}H and ^{13}C) is a modest experimental effort and will give immediate information on the purity of the sample and some general information on its structure. The structural information obtained from different NMR methods are summarized below.

In oligoglycosides, the glycosylation causes a down field shift of 4.2- 8.5 ppm of the α-carbon, the hydroxyl of which has been directly involved in the glycosylation while neighboring β-carbon atoms show an upfield shift of 0.5-2.0 ppm. These α- and β-shifts are independent of the nature of the monosaccharide and provide a conductive method for the establishment of interglycosidic

linkages. Anomeric configuration is determined by the one bond ^{13}C-^{1}H coupling constant for the anomeric carbon which strictly depends upon the orientation of the anomeric hydrogen.

The number of anomeric signals determines the number of monosaccarides, while best fit matching with appropriate sugars lead to their identification.

Furanose sugars are readily distinguished from their pyranose isomers as these differ significantly in the chemical shifts for C-1, C-2 and C-4 (downfield) and C-5 (upfield) in the furanose form. The sequence of sugars in a saponin can be predicted on the basis of chemical shifts as well as by determination of relaxation time (T_1) measurements. Site of the sugar linkage with the aglycon can be obtained by a comparison of the chemical shifts of the sapogenin with those of the saponin as glycosylation of a hydroxyl aglycon causes a change in the chemical shift due to the oxy- group modification. This leads to the down field shift of α-carbon atom and up field shift of the adjacent carbon atoms[32].

An extensive compilations, with an excellent discussion pertaining to the ^{13}C chemical shifts for various categories of natural products has been presented by Wehrli and Nishida[33]. A systematic compilation of the ^{13}C-NMR chemical shifts for around 400 steroidal derivatives has been published by Hunt and Stothers[34]but which includes only eight steroidal sapogenins. Tsuda and Schropfer[35] discussed the ^{13}C-NMR shielding behaviour for the olefinic carbon in a variety of steroidal sapogenins.

In general ^{13}C NMR spectra are recorded under proton-noise (broad band) decoupling in order to avoid the severe signal overlap due to large one bond ^{13}C-^{1}H coupling constants (*ca*-120-250 Hz). For single frequency off resonance decoupled (SFORD) spectra[36] the decoupler frequency is positioned outside the ^{1}H couplings are reduced to give rise to small residual couplings (J_r) from which the number of attached hydrogens can be determined (singlet for quaternary carbon, doublet for methine, triplet for methylene and quartet for methyl groups). However, the reduced coupling pattern still exhibits so much overlap that only a few of the carbon signals can be confidently assigned.

DEPT (Distortionless Enhancement by Polarization Transfer) (Doddrell et al. 1982) has been developed for discriminating carbon types, particularly CH from CH_2 and methyl in the cases where their signals are overlapped in the ordinary SFORD spectrum. Information for signal assignment can be obtained by comparing the chemical shifts of the original compounds with those of its derivatives. Acetylation has been successfully used at least for the assignment of the hydroxyl bearing carbon and adjacent carbon atoms as it shifts the α carbon downfield (2.3 ppm) with a concurrent upfield shifts (2.0-5.0 ppm) of the β carbon atoms while other signals remain almost unaffected. The β-upfield shifts are reasonably attributed to a γ-effect of the carbonyl carbon, since the free rotation of the acetate group produces conformation where the carbonyl carbon is in gauche position with respect to the β carbon atoms. Acetylation of homoallylic alcoholic function results in a down field shift (2.2-2.5 ppm), and an up field shift (3.8-4.2 ppm) of the β carbon.

Permethylation followed by hydrolysis experiments to determine the sugar sequence and the site of interglycosidic linkage has been widely employed for the structure establishment of saponins having triterpenoids, steroids or steroidal alkaloids as the sapogenin moiety[17]. Hydrolysis and other cleavage methods amenable to oligosaccharide analysis have been recently reviewed[37]. This whole process again is somewhat tedious and also consumes oligosaccharide, which is often very difficult to separate and purify and may be better employed in biological evaluation experiments.

The 1D ^{1}H-NMR spectrum of a glycoside showed only some recognizable signals, such as anomeric protons at 4.3- 5.9 ppm, methyl doublets of 6- deoxy sugar residues at 1.1- 1.3 ppm and various other protons with distinctive chemical shifts. The vast majority of proton resonances appear in very small spectral region which of 3.0-4.2 ppm, with subsequent problems. These derive from the bulk of non-anomeric methine and methylene protons which have very similar chemical shifts in different monosaccharide residues. Resonances at lowest field (4.8-5.3) ppm which are doublets with $^{3}J_{1.2}$ in the range 1-4 Hz are those of α-anomeric protons, whereas β-anomeric protons appear as doublets between 4.4-4.8 ppm with $^{3}J_{1.2}$ in the range 6-8 Hz in monosaccharides with gluco and galacto stereochemistry.

In pyranosides, the six membered rings generally form a chair of fixed conformation providing a classification of proton as axial or equatorial. If the H-2 is

Full Paper

axial, as it is for gluco and galacto tereochemistry, then a small coupling constant ($^3J_{HH}$) of ca 2-4 Hz is observed as a result of the gauche conformation of H-1 and H-2. The trans diaxial relationship of H-1 and H-2 in β-anomers of sugars with a gluco and galacto configuration leads to larger (7-9 Hz) coupling constants. Various 1D method such as spin decoupling; NOE (Nuclear Overhauser Effect), INDOR (Internuclear Double resonance) and partially relaxed spectroscopy have been used to unravel hidden resonances in the unresolved envelope. One individual resonance have been assigned to specific sugar residues, then NOE and relaxations experiments involving these resonances can help to determine linkage and sequence.

Although it might appear that the complete assignment of the ^{13}C-NMR spectrum of a carbohydrate residue is easier than that of a ^{1}H-NMR spectrum with its array of overlapping multiplets. The anomeric carbon signals resonate in a distinctive region 90-112 ppm. This is true for O-glycosides only and anomeric resonance in the case of C- glycosides, being monooxy substituted appear in the chemical shift range 70-80 ppm. The C-1 of reducing end residue usually appears in the region 90-98 ppm and C-1 of O-linked carbohydrates (non reducing monosaccharides appear at 98-112 ppm; hence the degree of oligomerization can be predicted. The rest of the methine methylene resonances absorb between 51 and 86 ppm.

The multiplicity of carbon signals is a valuable aid to spectral assignment. DEPT (distortionless enhancement by polarization transfer) and INEPT (insensitive nuclei enhanced by polarization transfer[38] pulse sequences involve transfer of polarization form ^{1}H to ^{13}C and are useful for distinguishing between methine, methylene and methyl carbons, each of which is directly coupled to a different number of protons. Such information is particularly valuable for establishing the structure of appended groups, which orrespond to the aglycon in glycosides. The substitution at a sugar ring by another sugar unit induces chemical shift changes in ^{1}H and ^{13}C NMR spectra, referred to as glycosylation shifts. It also helps in determining the presence of sulfate group.

2D NMR is more useful in determining the structure of saponins. One-dimensional NMR methods yield limited information for the determination of the complete structure and stereochemistry of glycosides including complex saponins. For the interpretation of ^{1}H NMR spectra of saponins which are not identical to those closely related to known compounds, complete assignment of the methine and methylene resonances in the poorly resolved groups of signals in the region of 3.2- 4.0 ppm adds greatly to the structural information.

Composed mainly of linear chains of coupled spin carbohydrates are especially suited to spin correlation methods such as decoupling or COSY and related techniques for identification of all the protons present in a given sugar residue. These COSY spectra contained informations on spin coupling networks within the constituent residues of the monosaccharide through the observation of cross peaks. COSY are of many types such as PS-COSY[39], DQF-COSY[40] TQF-COSY[41], RELAY COSY, and TOCSY.

NOESY which depends on proton proximity, can thus be a valuable assignment aid and in the assessment of its molecular conformation (i.e. 3D structure). NOEs are primarily used for determination of the sequence of sugar residues and also in determining their linkage positions. In HETCOR spectrum, each cross peak arises from connectivity between a ^{13}C nucleus and its directly bonded proton having the coordinates (δC, δH). HETCOR are of many types such as HMQC-COSY, HMQC-RELAY etc. Long-range heteronuclear chemical shift correlations such as COLOC and HMBC[42] are very sensitive techniques for establishing glycosidic linkages and other information.

Excellent comprehensive reviews on the triterpenoid saponins by Rastogi and his co-workers[43,20] covered the literature upto 1978. An excellent review on the chemistry and biological significance of saponins in foods and feeding stuffs was published[44]. Another review[45] briefly reviewed the advances in the structure elucidation of triterpenoid saponins.

Sterols

In the sea cucumbers, Δ^7-sterols, are exhibited probably as a consequence, presence of the haemolytic saponins, whereas in other Echinoderms, usual Δ^5-sterols[46] are presented. Saponins showed a much lower affinity for Δ^7-sterols and this helped to explain the apparent immunity of sea cucumbers towards their own saponins[47]. The sterol mixture from holothurians showed selenium dioxide test and prominence of the

830 cm^{-1} IR band, which is characteristic of a Δ^7 double bond[48].

Ceramides and gangliosides

Ceramides are amides of fatty acids with long-chain di- or trihydroxy bases, the commonest in animals being sphingosine and in plants phytosphingosine. The acyl group of ceramides is generally a long-chain saturated or monounsaturated fatty acid. The most frequent fatty acids found in animal ceramides are 18:0, 24:0 and 24:1(n-9), however long-chain hydroxy fatty acids have also been isolated. Free ceramides have been found in small amounts in animal tissues (the stratum corneum of the skin is exceptionally rich in ceramides) and they are now considered as lipid messenger molecules with an emerging role in growth suppression and apoptosis (cell death). Ceramides are the simplest sphingolipids and situated at the center of sphingolipid metabolism. Thus, the transfer of a phosphorylcholine head group from phosphatidylcholine to ceramide yields another phospholipid, sphingomyelin (also sphingolipid) and the addition of carbohydrate groups from the sugar donor, UDP-hexose, yields complex glycosphingolipids (cerebrosides, sulfatides, gangliosides). These compounds can be converted back to ceramide by the removal of sugars (glycosidases) or phosphorylcholine by sphingomyelinases. An enzyme (ceramidase) is able to cleave the amide-linked fatty acid of ceramide and free sphingosine.

Gangliosides are the group of glycosphingolipids that showed the greatest structural variation and also the more complex structure. These glycosphingolipids were discovered in 1942. After their isolation from brain tissue (they account for about 6% of the lipid weight) and identified sphingosine, fatty acid, hexose and a substance, which was called neuraminic acid, and which gave a purple color with Bial's reagent. This acid was later identified to be the same compound as the sialic acid isolated by Blix from mucin.

Gangliosides are glycosphingolipids that contained the structure of a ceramide plus carbohydrate moieties. Sialic acid is a part of the carbohydrate component. Gangliosides differ from cerebrosides in containing sialic acid, whereas the latter contain no sialic acid. Gangliosides are sialoglycosphingolipids. They are named as *N*-acetyl- or *N*-glycoloylneuraminosyl derivatives of the corresponding neutral glycosphingolipid. The position of the sialic acid residue(s) is indicated in the same way as is the case of a branched structure.

Miscellaneous

Seacucumbers possess a peculiar specialized defense system: the so-called Cuverian tubules. When the animal is mechanically stimulated, a few white filaments (adhesive) are discharged. This adhesive material consists of 60% proteins and 40% carbohydrates, a compound that is unique among the adhesive secretions of marine invertebrates. Although it is highly insoluble, a small fraction of this material can be extracted using denaturing buffers. Electrophoretic analysis of the extracts revealed that it contains about 10 proteins with apparent molecular masses ranging from 17 to 220 K.daltons and with closely related amino acid compounds, rich in acidic and in small side chain amino acids[49]. Sea cucumbers also contained mucopolysaccharides, chondroitins, protein, vitamins A and C, riboflavin, niacin, calcium, iron, magnesium, zinc, sodium, and carbohydrates. The nutrient content of sea cucumbers is extremely broad, and total identification of each innate constituent will probably never be established. The University of Queensland Centre for Drug Design conducted a series of chromotographs that displayed "an enormous number of compounds". Some of the compounds identified unique to the animal kingdom include two types of triterpenoidal oligoglycosides, one type occurring with a sulfate group and the other without. Molecules of this general classification are abundant in the plant kingdom, but are quite rare among animal tissues. Other compounds found in abundance include analogues of monosaccharide structure and analogues of sulfated mucopolysaccharide configuration.

Sea cucumber has shown an ability to balance prostaglandins, which regulate the inflammatory process. Sea cucumber has a cartilaginous body that serves as a rich source of mucopolysaccharides; mainly chondroitin sulfate, known for its ability to reduce arthritis pain. Chondroitin's action is similar to that of glucosamine sulfate, the main building block of chondroitin.

Chondroitin building blocks can be repeated numerous times. This is basically a glucose molecule (left portion) and glucosamine molecule (right portion), which has been sulfated (O_3S, at the top). Long-chain sul-

Full Paper

fated polysaccharides, like chondroitin, also inhibit viruses.

Echinoderms are rich both in quality and quantity of polyunsaturated fatty acids[50]. An anti HIV pharmaceutical contains polyfucose sulfate and or salts thereof derived from *Holothuria*ns. The polyfucose sulfate consists of hexosamine 1-7, fucose 45-65 and sulfate 25-42% by weight, and molecular weight is 20,000-200,000[51]. A detailed presentation of the structures of secondary metabolites of Echinodermata and their significance as chemotaxonomic markers can be found in the article by Stonik and Elykov[15].

Activities reported

Most saponins exhibit potent hemolytic properties[52], antitumor[53] anti-inflammatory[8] antibacterial[54] activities. Recently, several triterpenoid glycoside sulfates have been isolated from the sea cucumber *Cucumaria echinata* (Cucumariidae) and its corresponding aglycones showed activity against L-210 and KB cells[55]. Due to the general toxicity Of the saponins it is generally believed that these compounds act as chemical defense agents. The toxins are concentrated in specialized organs knows as Cuvier glands which can be eviscerated to deter predators.

It is interesting to note that echinoside-A had a significant inhibitory effect against Na^+, K^+- adenosine triphosphatase (Na^+, K^+– ATPase.)[56]. Echinoside A showed antifungal activity and a broad spectrum of antimicrobial activities & was effective in treatment of athlete's foot[57].

A review pertaining to antibotic properties[58] of triterpene glycosides from Holothuroidea has been published. Holotoxin A and B are noteworthy because of their distinct growth inhibitory[59] activities against pathogenic microorganism eg. *Tricophyton* sp., *Candida* sp., and *Trichomonas* sp.,Antibacterial and antifungal activities of alc. extract of holothurian species such as *Actinopyga echinities, A. miliaris, Holothuria atra,* and *H. scabra* of Tamil Nadu coast was studied. Bacteria such as *Aeromonas hydrophila, Escherichia coli, Enterococcus sp., Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Vibrio harveyi* and fish borne mold, *Aspergillus sp.* were inhibited at varying levels by the extract of *A. miliaris, H. atra* and *H. scabra. Bacillus sp.* was not affected by holothurian extracts. The results of the study revealed the presence of antimicrobial substances possibly steroidal sapogenins in holothurian. These exists a great potential for the extract of bioactive substances of medical importance[60] at a exploitable cost from these holothurians.

Several lanostane type triterpene oligoglycoside in sea cucumber exhibit antifungal activities. Those sea cucumber saponins are actually holothurins from *S. japonicus* and *H. leucospilota,* echinosides A and B from *A.echinetes,* holothurin A and 24dehydroechinoside A from *A. agassizi,* and bivittosides A, B, C and D from *B. bivittata*. Sea cucumber *H. pervicax* which is closely related species to *H. leucospilota* and inhabits the Indo Pacific coast, contained three saponins, name pervicoside A, pervicoside B and pervicoside C. Their desulfated form that is DS-Pervicoside A, DS. pervicoside B and DS pervicoside- C exhibited distinct antifungal activities against various pathogenic microorganisms. DS pervicoside B was most active[61]. Triterpenoid glycosides from *Stichopus chloronotus, Bohadschia marmorata, Bohadschia sp., B. argus* and *B. graeffi* (Holothuridae) (3-100 mu.g/ml) exerted stronger fungistatic action against *Candida albicans, C. tropicalis, C. utilis,* and *C.krusei* than did similar glycosides from 10 other species of pacific holothurians[62]. Glycosides of holothurians (holothurin-A and holothurin-B from *H. mexicana,* stichoposide A and stiochoposide C from the family stichopodidae and cucumarioside G from *C.fraudatrix*) showed a higher toxicity. Their cytotoxic activity depended on the number of monosaccharide units, attached to the hydroxy group at C-3. Holothurin A had four such units, while Holothurin B had only 2, this was reflected in the decrease in cytotoxicactivity. On the other hand, the increase in the length of saccharides chain to 6, had little effect on activity. The changes noted indicate the general tendency of these glycosides to have increased physiological activity[63] in response to an increase in length of the chains to 4-6 monosaccharide residues

Two fucose-containing acidic polysaccharides, HL-S and HL-P, were obtained from dried body wall of *H. leucospilota*. Both polysaccharides were pure by electrophoretic and gel chromatography as well as ultracentrifugal analysis. According to anal. and phys. Data.

HL-S seemed to be polyfucose sulfate, which was found in most sea cucumber and HL-P was identified as a sulfated mucopolysaccharide consisting of galactosamine, glucuronic acid, fucose, and sulfate with the molar ratio of1:0.94:0.84:3.60 respectively. HL-P was a powerful inhibitor of thrombin[64] *in vitro* and also showed significant inhibitory effects on tumor growth in mice.

The newly isolated cerebrosides C E-1-1, C E-1-2, C E-1-3 and C E-3-2 from *Cucumaria japonica* have been tested for their lethality towards brine shrimps at a 30 ppm concentration according to the brine shrimp lethality bioassay. They exhibit lethal rates of 27,11,31,19 and 22% respectively: On the other hand, the ganglioside molecular species, CG-1 from C. japonica, is found to exhibit neuritogenic activity toward the rat pheochromocytoma cell line, PC-12 cells. The isolation and characterization of the biologically active glycosphingolipids is attracting considerable attention with regard to the manufacture of new medicines from marine natural products.

The gangliosides HPG-1, HPG-3 and HPG-8 showed neuritogenic activities (Anisimov et al. 1980) toward the rat pheochromocytoma cell line PC-12 cell at 10 mu/ml *in vitro*.

It is known that GSLs have numerous physiological functions due to variations in the sugar chain, in spite of very small constituents. Those are classified into cerebrosides, sulfatides, ceramide oligohexosides, globosides and gangliosides based on the constituent sugars. Gangliosides, sialic acid containing GSLs are especially enriched in the brain and nervous tissues and are involved in the regulation of many cellular events. Recently a number of GSLs have been isolated from sea cucumber. The research is going on that biological active GSLs from echinoderms to elucidate the structure- function relationships of GSLs and to develop novel medicinal resources. The characteristics of GSLs from sea cucumber and structure- activity relationships had neuritogenic activity towards the rat pheochromocytoma cell line PC-12. That is most of the cerebroside constituent of sea cucumber are same glucocerebroside as in other animals, while the ganglioside constituents were unique in that a sialic acid directly binds to glucose of cerebroside, they are mutually connected in tandem and some are located in the internal parts of sugar chain. It also became apparent sialic acid is indispensable for the neuritogenic activities[65]. Three ganglioside molecular species, SCG-1, SCG-2, and SCG-3 were obtained from the lipid fraction of the $CHCl_3$-MeOH extract of sea cucumber *Stichopus chloronotus*. Moreover, these three gangliosides exhibited neuritogenic activity toward the rat pheochromoctoma PC-12 cells in the presence of nerve growth factor. The gangliosides HLG-1, HLG-2 and HLG-3 from *Holothuria leucospilota*, display neuritogenic activity on the neuritogenesis of the rat pheochromocytoma cell line, compared with H_2O (control) at a concentration of 10 μm in the presence of NGF (5μg/ml). The isolation and characterization of such neuritogenetically active gangliosides is thus attracting. Considerable attention with regard to the manufacturing of new medicines from marine natural productsthus be further explained.

Holothurinosides A, C and D from *Holothuria forskali*, showed antitumor activity against P388, A549, HeLa and B-16 cells *in vitro*. These compounds also showed some antiviral activities[66].

ACKNOWLEDGEMENTS

Financial assistance in the form of Emeritus Scientist to VL is gratefully acknowledged to the HRDG, Council of Scientific and Industrial Research, Government of India, New Delhi, India which helped in compiling this work. We are also grateful to the Director CSIR- Central Drug Research Institute for providing an excellent libraryfacilities.

REFERENCES

[1] D.B.James, Naga; InTwenty Sea Cucumbers from Seas around India,The ICLARM Quarterly, **24**, 408 **(2001)**.

[2] E.N.Anderson; The food of China, New Haven, CT: Yale University, **(1988)**.

[3] Z.Enchin; Chinese medicated diet. Shanghai: Publishing House of Shanghai College of Traditional Chinese Medicine, **(1988)**.

[4] I.Hawa, M.Zulaikah, M.Jamaludin, A.A.Zainal Abidin, M.A.Kaswandi, B.H.Ridzwan; The potential of the coelomic fluid in sea cucumber as an antioxidant, Malasian Journal ofNutriton, **5**, 5-59 **(1999)**.

Full Paper

[5] C.Jiaxin; Overview of sea cucumber farming and sea ranching practices in china, SPC Beche-de-mer Information Bulletin., 18-23 **(2003)**.

[6] W.Fangguo; Nutrient analysis of frozen sea cucumber (Acaudina molpadioidea), Donghai Marine Science., **15(4)**, 5-67 **(1997)**.

[7] F.Huizeng; Sea cucumber: Ginseng of sea, Zhongguo Marine Medicine. **82(4)**, 37 **(2001)**.

[8] L.A.Goldsmith, G.P.Carlson; Proceedings 1974 edited by H.H Webber and G.D.Ruggieri. Matrine technology society, Washington D.C., In Food and Drugs from the Sea, 354-365 **(1974)**.

[9] G.Bakus; Defensive mechanisms and ecology of tropical holothurians, Journal Marine Biology, **2**, 23-32 **(1968)**.

[10] G.B.Elyakov, V.A.Stonik, E.V.Levina, V.P.Slanke, T.A.Kuznetsova, V.S.Levin; Glycosides of marine invertebrates-I. A comparative study of the glycosides fraction of Pacific sea cucumbers.Comp.Biochem.Physiol., 44, 325-336 **(1973)**.

[11] T.Yamanouchi; On the poisonous extracts from holothurians (in Japanese) TeikokuGakushiinHokoku 73 **(1942)**.

[12] R.F.Nigrelli, The effects of holothurian on fish, and mice withsarcoma, Zoologica, NewYork, **37**, 89-90 **(1952)**.

[13] J.D.Chanley, R.Ledeen, J.Wax, R.F.Nigrelli, H.Sobotka, I.Holothurin; The isolation, properties and sugar components of holothurin A, J.Am.Chem.Soc, **81**, 5180-5183 **(1959)**.

[14] D.J.Burnell, J.W.Apsimon; In Marine Natural Products, Echinoderm saponin Chemical and Biological Perspectives (Chemical and Biological Perspectives) Academic Press, New York, **5**, 287-389 **(1983)**.

[15] V.A.Stonik, G.B.Elyakov; Secondary Metabolites from Echinoderms as Chemotaxonomic Markers. In Bioorganic Marine Chemistry (Scheuer,P.J.,ed.) BerlinHeidelberg New York Tokyo: Springer, 43 **(1988)**.

[16] S.Shibata; In New natural Products and Plant Drugs with Pharmacological,Biologcal or Therapeutical Activity (H.Wagner, P.Wulff, (Ed),Springer, Berlin, 177 **(1977)**.

[17] G.Wulff, R.Tschesche; Chemistry and biology of saponins.In Forscheritte der Chemie Organischer Naturstoffen, **30**, 461 **(1973)**.

[18] G.Blunden, M.C.Culling, K.Jewers; Steroidal sapogenins: a review ofactual and potential plant sources, Trop.Sci., **17**, 139-154 **(1975)**.

[19] K.Hiller, G.Voigt; Neue ergebnisse in der Erforschung der triterpene saponine. Die Pharmazie, **32**, 365-393 **(1977)**.

[20] R.S.Chandel, R.P.Rastogi; Triterpenoid Saponins and Sapogenins,Phytochemistry, **19**, 1889-1908 **(1980)**.

[21] S.B.Mahato, A.N.Ganguly, N.P.Sahu; Steroid saponins, Phytochemistry, **21**, 959-978 **(1982)**.

[22] S.B.Singh, R.S.Thakur; In Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins,J.Sci.Ind.Res., **42**, 319 **(1983)**.

[23] K.Hostettman, M.K.Hostettman, K.Nakanishi; Droplet counter-current chromatography for the preparative isolation of various glycosides, J.Chrom, **170**, 355-361 **(1979)**.

[24] S.B.Mahato, A.K.Nandy; Triterpenoid saponins discovered between 1987 and 1989, Phytochemistry, 1357-1390 **(1991)**.

[25] H.R.Schulten; Field Desorption Mass Spectrometry of Oligosaccharides, Int.J.Mass Spect.Ion Phys, **32**, 97-283 **(1979)**.

[26] S.B.Singh, R.S.Thakur, H.R.Schulten; Spirostanol saponins from *Paris polyphylla*, structures of polyphyllin C, D, E and F, Phytochemistry, **21**, 2925-2529 **(1982)**.

[27] M.Barber, R.S.Bordoli, G.J.Elliott, R.D.Sedgwick, A.N.Tyler; Fast atom Bombardment mass spectrometry. Anal.Chem., **54**, 645 **(1982)**.

[28] H.R.Schulten, S.B.Singh, R.S.Thakur; InA NewApproach to Accurate Mass measurement by Field Desorption peak MatchingMass Spectrom Soc.Jpn, Z.Naturforsch, **39,** 201 **(1984)**.

[29] P.K.Agarwal, NMR Spectroscopy in the structural elucidation of oligosaccharides and glycosides, Phytochemistry, **31 (10**), 3307-3330 **(1992)**.

[30] I.Kitagawa, M.Yoshikawa; YImakura and I Yoshioka, Saponin and sapogenol, Chem.Pharm.Bull., **22**, 1339-1347 **(1974)**.

[31] F.Smith, A.M.Unrau; The Structure oj. Laminarin (Doctoral thesis, Univ.Minnesota, Chem.Ind., 881 **(1959)**.

[32] P.K.Agrawal, D.C.Jain, R.K.Gupta, R.S.Thakur; Carbon-13 NMRspectroscopy of steroidalsapogenins and steroidal saponins, Phytochemistry, **24**, 2479-2496 **(1985)**.

[33] F.W.Wehrli,T.Nishida; In Progress in the Chemistry of Organic Natural Products (Forscheritte der Chemie Organischer Naturstoffen), Springer,Vienna, **36**, 1 **(1979)**.

[34] J.W.Blunt, J.B.Stothers; In Organic Magnetic Resonance, **9**, 430 **(1977)**.
[35] M.Tsuda, G.J.Schroepfer; Olefinic Carbon Shieldings in Sterols and Relate CyclicCompounds, Chem.Physiol.Lipids, **25**, 49-68 **(1979)**.
[36] J.B.Stothers; In Carbon-13 NMR spectroscopy, Academic Press. New York, 149 **(1972)**.
[37] C.Biermann; In Advances inCarbohydrates Chemistry and Biochemistry, **47**, 45 **(1988)**.
[38] D.P.Burum, R.R.Ernst; Net polarization transfer via enhancement oflow-sensitivity nuclei, J.Mag. Reson., **39**, 163-168 **(1980)**.
[39] A.Bax, M.F.Summers; ^{1}H and ^{13}C Assignments from Sensitivity Enhanced Detection of Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum NMR J.Am.Chem. Soc., **108**, 2093-2094 **(1986)**.
[40] M.Rance, O.W.Sorensen, G.Bodenhausen, G.Wagner, R.R.Ernst, K Wuthrich,Improved spectralresolution in cosy 1H NMR spectra of proteins via double quantum Filtering, Biochem.Biophys.Res.Comm., **117**, 479-485 **(1983)**.
[41] U.Piantini,O.W.Sorensen, R.R.Ernst; Multiple quantum filters forelucidating NMR coupling networks,J.Am.Chem.Soc., **104**, 6800-6801 **(1982)**.
[42] A.Bax, M.F.Summers; ^{1}H and ^{13}C Assignments from Sensitivity EnhancedDetection of HeteronuclearMultiple Bond Connectivity by 2D Multiple Quantum NMRJ.Am.Chem.Soc.,**108**, 2093-2094 **(1986)**.
[43] S.K.Agarwal, R.P.Rastogi; Triterpenoid saponin and their genins,Phytochemistry, **13**, 2623- 2645 **(1974)**.
[44] K.R.Price, I.Johnson, G.R.Fenwick; The chemistry and biological Significance of saponins in foods and feeding stuffs, Critical Reviews in FoodScience and Nutrition, **26**, 27-135 **(1987)**.
[45] P.Cai; Advances in structure elucidation of triterpenoid saponins Yaoxue Tongbao, **17**, 668-674 **(1982)**.
[46] L.J.Goad; In Marine Natural Products, Chemical and Biological Perspectives (Scheuer,P.J.,ed.), New York: Academic Press, **2(75), (1978)**.
[47] D.J.Burnell, J.W.Apsimon; In Marine Natural Products, Echinoderm saponins Chemical and Biological Perspectives (Chemical and Biological Perspectives) Academic Press, New York, 287-389 **(1983)**.
[48] K.Skai, K.Tsuda; In Sterols of Echinoderms, (By K.C.Gupta 1968 Elsevier) Chem.Pharm.Bull, **11**, 529-539 **(1963)**.
[49] S.D.Moore, J.W.Herbert, J.J.Michel, J.F.Patrik; In Marine Biotechnology, **5(1)**, 45 **(2003)**.
[50] S.V.Isay, N.G.Busarova; Study on fatty acid composition of marine Organisms-I Unsaturated fatty acids of Japan Sea invertebrates, Comp.Biochem.,**77(4)**, 803-810 **(1984)**.
[51] Taiho Pharmaceutical Co.Ltd., Kotai Kasei Co.Ltd. PCT Int Appl.WO, **92(02)**, 231**(1992)**.
[52] L.J.Goad, F.X.Garneau, J.L.Simard, M.Girard; Composition of the free, esterifiedand sulphated sterols of the sea cucumber Psolus fabricii,Comp.Biochem Physiol., **84B**, 189-196 **(1986)**.
[53] Y.Hashimoto, Marine Toxins and Other Bioactive Marine Metabolites, Japan ScientificSocieties Press, Tokyo, 268-288 **(1979)**.
[54] G.R.Pettit, J.FDay, J.L.Hartwell, H.B.Wood; Antineoplastic Components of6 Marine Animals, Nature, **227**, 962-963 **(1970)**.
[55] G.D.Ruggieri, R.F.Nigrelli; Physiologically Active Substances fromEchinoderms. In: H.J.Humm, C.E.Lane, (Eds); Bioactive Compounds from the Sea, Marine Science Series , Marcel Dekker, New York., **1**, 183-195 **(1974)**.
[56] T.Miyamoto, K.Togawa, R.Higushi, T.Komori Tand Sasaki; Six newlyidentified biologicallyactive triterpenoid glyco- side sulfates from the sea cucumber *Cucumaria echinata*. Liebigs Annals Chemistry, 453-460 **(1990)**.
[57] I.Kitagawa, M.Kobayashi, M.Fuchida, Y.Kyogoku; Marine natural products. XIV. Structures of echinosides A and B, antifungal lanostane-oligosides from the sea cucumber Actinopyga echinites (Jaeger).Chem.Pharm.Bull, **33(12),** 5214-5224 **(1985)**.
[58] Takeda Chemical Industries Ltd.Jpn., Kokai Tokkyo Koho Jp, **57(212)**, 200 **(1981)**.
[59] A.V.Samoilov, E.S.Grshovich; Antibiotic properties of the triterpenes glycosides from marineanimals of the class Holothuridea, Antibiotiki, **25(4)**, 307-313 **(1980)**.
[60] I.Kitagawa, T.Nishino, T.Sugawara, Saponin and sapogenol. XXVTI. Revised structures of holotoxin A and holotoxin B, two antifungal oligoglycosides from sea cucumber *Stichopus japonicas*,Chem.Pharm.Bull., **26**, 3722-3731 **(1978)**.
[61] A.T.Jawahar, J.Nagarajun, S.A.Shanmugan; Antimicrobial substances of potential

biomedicalimportance from Holothurians species, Ind.J.Mar. Sci., **31(2)**, 161-164 **(2002)**.

[62] I.Kitagava, M.Kobayashi, B.W.Son, S.Suzuki, Y.Kyogoku; Pervicosides A, B,and C, Lanostane-Type Triterpene-Oligoglycoside Sulfates from the Sea Cucumber Holothuria pervicax., Chem.Pharm.Bull, **37**, 1230-1234 **(1989)**.

[63] N.S.Sarma, A.S.R.Anjaneyulu, C.B.S.Rao, V.Venkateswarlu; Triterpene Glycosides and Glycosides of Sea Cucumbers Holothuria-atra and Scabra (Holothurideae), Ind.J.Chem., **26B**, 715-721 **(1987)**.

[64] M.M.Anisimov, V.V.Shcheglov, V.A.Stonik, A.L.Kul'ga, Z.V.Levina, V.S.Levin, G.B.Elyako; Comparative study of the antifungal activity ofTriterpenic glycosides of Pacificholothurians. Doklady Akad Nauk SSSR., **207(3)**, 711-713 **(1972)**.

[65] F.Huizeng, C.Judi, L.Peihong, H.Xiaog, Haitang; In Sulphated polysaccharides pharmaceutically accepted sats thereof, Yaoxue Xuebao, **18(3)**, 203-208 **(1983)**.

[66] K.Yamada, K.Sasaki, Y.Haroda, R.Ibose, R.Higuchi; Constituents of holothuroidea, 12. Isolation and structure of glucocerebrosides from the *sea cucumber Holothuria pervicax*, Chem.Pharm. Bull., **50(11)**, 1467-1470 **(2002)**.