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Review Article

Acanthaster planci Crown of Thorns (Starfish) - A Predatory Marine Invertebrate with a Commercial Wealth Viable Biomass of Natural Product Lead Compounds.

Farid Che. Ghazali[1], Alias A. Karim[2], Salizawati Muhamad Salhimi[3], Sirajudeen, K. N. S[4], Hisham Atan Edinur[1], Nur Hanim Zainudin[1]

[1]School of Health Sciences, USM Health Campus, 16150, Kelantan, Malaysia
[2]School of Industrial Technology, Universiti Sains Malaysia (USM), School of Industrial Technology, Universiti Sains Malaysia, 11800 USM Penang, Malaysia
[3]School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM), School of Industrial Technology, Universiti Sains Malaysia, 11800 USM Penang, Malaysia
[4]School of Medical Sciences, USM Health Campus, Kubang Kerian, 16150, Kelantan, Malaysia

***Corresponding Author:**
Farid Che. Ghazali
Email: farid@usm.my

Abstract: Increasing interest for availability of tangible, novel, added value and sustainable commercial exploitable of nature therapeutic compounds including those derived from marine sources have enriched a long history of research into *Acanthaster planci* Crown-of-thorns (COT) starfish biology, ecology, and the causes of their outbreaks. These are important aspects for a sustainable commercial optimization and exploitation of *Acanthaster planci* COT especially for health related agendas or therapeutic application. This manuscript thus seeks to outline present and future research strategy and works pertinent to *Acanthaster planci* COT locally and global focused on evidence-based optimised sustainable exploitation as well as refining management actions to improve the performance of control programs. Among deuterostomes, the regenerative potential is maximally expressed in echinoderms such as the *Acanthaster planci* COT; these animals can quickly replace most injured organs. In tandem to that, views for further gene related molecular characterization and chemical analysis of potential cell mediated protein from these marine invertebrates that have potential in medical and pharmaceutical therapeutics will be highlighted.

Keywords: Crown of thorns starfish, marine invertebrates, biomass and molecular screening, Therapeutic applications.

INTRODUCTION

Globally, there is an increasing interest for availability of tangible, novel, added value; sustainable commercial exploitable therapeutic compounds of naturally derived resources. In this context: the oceans cover more than 70% of the world surface with more than 300,000 known species of fauna and flora. It is reported that these marine biodiversity is so vast that the total majority of phyla and more than 90% of all living classes of organisms are localized within the marine ecoenvironment. It is an important question to ask whether these marine derived natural products are actually tangible drug molecules. *Per se*, several years ago [1, 2], it was shown that those alkaloids which are presently available as pharmaceutical agents are a good fit (with some outlying molecules) to Lipinski's "rule of five". When an analysis of 120,000 compounds in the *Dictionary of Natural Products* was conducted, 65% had no violations of these rules, and this led to the development of a natural product library of over 500 compounds for drug screening [3]. Other studies have supported these conclusions [4]. Thus existing natural products can form the basis of a drug discovery program complementing the development of potential new sources of bioactive molecules. As such studies using these marine organisms as the source of new biologically active compounds have had a major influence on this millennium biomedical research [5]. Whereby these broad and structurally diverse arsenal of pharmacologically active natural products compound are said to be mainly accumulated inertly in marine invertebrates' anatomical biomass such as in sponges, tunicates, bryozoans, and molluscs. Some of these active compounds have been found to possess either novel or potent pharmacologic activities. In tandem to this, diverse peptides with a wide range of biological activities have been discovered, this including antimicrobial, antitumor, and antiviral activities and toxins. It is analysed that the chemical structure of these compounds is very different from those obtained from

terrestrial and microbial systems. As such it is thus possible to find cyclic and linear peptides and depsipetides (peptides in which one or more of the amide bonds are replaced by ester bonds) containing natural or non-natural amino acids. These molecules can be synthesized by the organism itself or can be obtained from marine microorganisms with whom they have a symbiotic relationship [6, 7]. At higher taxonomic levels extrapolation, most of these biological diversity is found either primarily or exclusively in the ocean. Of 33 modern phyla studied, only a few numbers were found within their terrestrial habitats while 28 phyla occur within the marine ecohabitats. Hence the diversity of life in the sea offers more possibilities for the discovery of organisms for use as models to explore various biological processes. Recent science also shows that the cumulative impact of human activities (such as coastal wetlands being drained and converted to upland habitat, fishing exploitation and industrialization pollution) have significant impact on ocean ecosystems [8, 9]. As such, studies have also shown that volume of biomass is purported to be continuing to increase exponentially in predatory fish populations for up to 18 years after reserves have been established [10].

Acknowledging the recent history of the relationship between humankind and the Earth, it is essential that the health care issues being left for our descendants be considered in terms of resources[11]. As said above, most of the exploitable marine based natural products are believed to be mainly accumulated in most local iconic geochemical signatures invertebrates' biomass such as the echinoderm, sponges, tunicates, bryozoans and molluscs. A serious obstacle and dilemma to the ultimate exploitation and development of these marine natural products is the problem of sustainable biomass supply. The concentrations of many highly active compounds in marine invertebrates are often minute, sometimes accounting for less than 10^{-6}% of the wet weight. For example, in order to obtain approximately 1 g of the promising anti-cancer agent ET-743, close to 1 metric tonne (wet weight) of the tunicate *E. turbinate* has to be harvested and extracted [12]. In other cases, such as for the halichondrins (e.g. halichondrin B), which are powerful cytostatic polyketides of sponge origin, the ratio of biomass to yield of product is even less favourable. In order to obtain as little as 300 mg of a mixture of two halichondrin analogues, 1 metric tonne of the sponge *Lissodendoryx* sp. had be collected and extracted [13].

In the context of Malaysia, folk medicines from geochemical signatures marine natural products are well practised. The marine environment has very few reported applications in traditional medicine. The red algae *Chondrus crispus* and *Mastocarpus stellatus* were sources of a beverage, which was popular as a folk cure for colds, sore throats, chest infections including tuberculosis. The alga was also boiled in milk or water and used for kidney trouble and burns [14, 15]. Furthermore, three spoonfuls of the juice of the red alga *Porphyra umbilicalis* (Linnaeus) Kützing, taken every morning followed by fasting for three weeks was found to be effective against cancers, in particular breast cancer [16]. *P. umbilicalis* has also been described in the Aran Islands for easing indigestion and was also boiled and given to cows to relieve their springtime constipation [17, 18]. As such some of these marine invertebrates are iconic features well exploited. Although the levels of their diversity within and between populations, sizes and demography are continuously monitored, however, very little scientific evidence is actually known of thus poorly understood on commercial exploitation able related tangibilities from the local geochemical signatures Malaysian COT biomass. Literature documentations are found wanting for supportive medical related knowledge or information in pertinent to whether or not bioactive ingredients are available from its inert biomass especially when compared to another echinoderm the sea cucumber. Geochemical signatures' sea cucumber, have various documentations that disclosure the potential these marine invertebrate enriched with mutable collagen and natural bioactive lead compounds of therapeutic potential such as sulfated polysaccharides glycosaminoglycans that is of potential as for harnessing wound healing patho-physiological cascade [19-21].

Present knowledge of pertinent regarding *Acanthaster planci* (COT) Starfish

Taxonomically, the *Acanthaster planci* COT is the second largest starfish categorized under the phylum. It is a carnivorous starfish that preys on the polyps of reef-building corals. The species is widely distributed across the Indo-Pacific in tropical and sub-tropical latitudes and occurs in most locations where scleractinian corals, the primary food of adult crown-of-thorns are common. Scleractinian corals are fundamental to the form and structure of coral reefs and therefore the structure and diversity of the communities of those reefs. As a consequence, high levels of COT predation on these corals have the potential to fundamentally alter both the reefs themselves and their biological communities. Knowledge on the taxonomy, phylogenetics, and phylogeography and population structure of a possible commercially important marine fisheries group/species is critical for informed decision for its management and conservation. Thus, species identification especially in closely related taxa in the *Acanthaster planci* COT is useful for appropriate management applications and sustainable conservation of its wild habitat biodiversity and ecosystem. This intertidal marine are named as starfish as they do not come under the category of vertebrates due to the lacking of an obvious anatomical spinal column. As such for this very reason they are classified under the class of marine invertebrates (See Table 1 below).

Table 1: *Acanthaster planci* COT starfish taxonomic phylogeny

Taxonomic ranks	Taxonomic hierarchy
Kingdom	Animalia
Phylum	Echinodermata
Subphylum	Eleutherozoa
Superclass	Asterozoa
Class	Asteroidea
Order	Spinulosida
Suborder	Leptognathina
Family	Acanthasteridae
Genus	Acanthaster
Species	*Acanthaster planci*

Adapted from Rowe and Gates, 1995.

It is a radially symmetrical marine invertebrate, with many short arms and numerous large, aboral spines, articulated on prominences from small basal ossicles [22, 23]. A well defined or prominent head anatomy is entirely absent. While individual coloration tends to varies from red and orange to purple, and is thought to be the result of differences in diet. This invertebrate also does have an exceptionally grown extent to of one meter in diameter. As such the average growth has been recorded to be of between 25cm to 35cm in length (Sikorski, 2006) (See Figure 1 below).

Fig-1: A digital photomicrograph topographical representation of the aboral surface (i) and oral surface (ii) of an adult COT starfish with several arms extension. Leica Ms5 stereomicroscope. 0.63 x magnifications

The aboral surface anatomy of COT body seems to appear to be quite indurate, but this feature still allows this invertebrate to bend and twist into all sorts of shapes to maneuver and fit itself to the contours of the coral substrate it feeds on. *Per se*, our microscopical characterization study of COT in Redang Island Malaysia, recorded an average mean body diameter measurement of 26.50 cm in length. While the average total number of arms per invertebrate was of the total mean of 16 arms per individual invertebrate. While the average bodily weight per invertebrate was of the mean weight of 490gram per individual COT starfish [24]. Topographically each individual COT invertebrates consisted of two major anatomical positions, a spiny (sharp protuberances coating its integumental) aboral surface or also known as the abactinal surface and an underlying oral surface (with a central based soft mucous filled opening). This flat oral located surface [which in natural condition remains towards the substratum], is a less pigmented surface and is also called the actinal surface. At the centre of this actinal surface is an aperture, the actinosome or the invertebrate mouth. Mucous secretion from this orifice have been purported to be of tangible medicinal valued. The more convex aboral surface forms the bodily central disk and numbers of projected arms with venomous spines. It's pertinent to acknowledge that the oral and aboral surfaces are not anatomically the said ventral and dorsal surfaces in other vertebrates' anatomy, but correspond to the left and right sides of the bilaterally symmetrical larva. This starfish is known to have a number of venomous spines on its body surface. These hinged spines have been measured up to 2-3 cm in length. Glandular cells are located in the epitheliums that cover each spine. These cells help to release toxic chemicals into the skin when the spine stings (Grzimek, 1972). [See figure 2 and 3 below for Confocal microscopy observation of the spine illustrated in photomicrograph figure to revealed high concentration of protein presence at the outer lining of the calcareous spine, suggesting high content of protein from the neurotoxin in the glandular epithelium] [24].

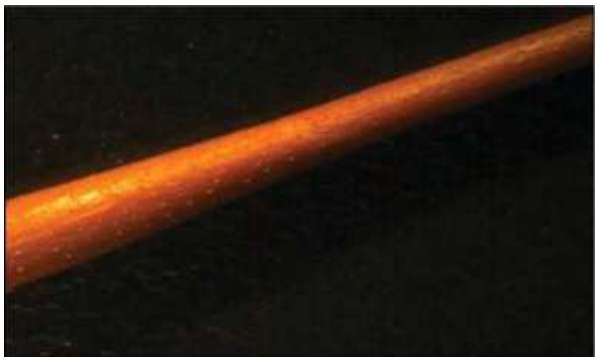

Fig-2: A photomicrograph of a single aboral located COT spine observed under Leica Ms5 stereomicroscope graph at 6.3 x magnifications. Note the surface is indented with sharp, protuberances fine structures

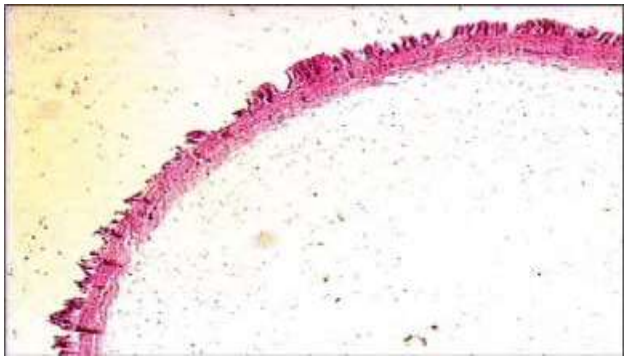

Fig-3: A magnification cross sectional photomicrograph of a single aboral located COT spine observed at 200 x magnifications. Note the Glomerular-like epithelium at its top surface. H&E stain

Tube-feet or podia which are actually thin-walled cylindrical tentacles are presence in COT. The anatomical organization of these podia in total and their combined efforts move the animal along the substrate. Coelomic cavities are present in starfish. This coelomic cavity is the anatomical internal structure of star fish which contains coelomic fluid holding the cells of immunity and the antimicrobial peptides. Antimicrobial peptides form the first line of defenses and hence termed as the host defense peptides. They come under the innate defense response in both unicellular and multi cellular organisms [25]. They have a wide range of activity towards bacteria, fungi, viruses and parasites. Antimicrobial peptides (AMPs) are vital immune effect or molecules for invertebrates, including echinoderms, which lack a vertebrate-type adaptive immune system.

Besides the anus located ventrally, there were also a number of madreporites, very small stony-like structures neighboring the central body and pairs of tiny hook-like spines called pedicellaria. The sensory biology of the crown-of-thorns starfish remains mostly unstudied. However three sensory modalities have been documented for other starfish: vision, olfaction and mechanosensation [26]. Photosensitivity in most echinoderms has been attributed to 'diffuse' dermal receptors. However there has been report that certain single calcite crystals used by brittlestars for skeletal construction are also a component of specialized photo-sensory organs, conceivably with the function of a compound eye. The analysis of arm ossicles in Ophiocoma showed that in light-sensitive species, the periphery of the labyrinthic calcitic skeleton extends into a regular array of spherical microstructures that have a characteristic double-lens design.

These structures are absent in light-indifferent species. It has been known for 200 years now that starfish have eye-like structures [27]. The eye consists of a thickening at the base of the unpaired tube foot at the very tip of the arm and resembles a simple compound eye. The function of the starfish eye has only recently been proven for one species of starfish, the Indo-Pacific Asteroidea starfish species blue starfish (*Linckia laevigata*), which uses its eyes for orientation towards coral reefs [28]. The light-sensitive eyespots (ommatidia) have been to be present at the tips of its projecting arms. These ommatidia are assumed (reasonably) used as vision organs. This starfish has simple compound eyes with about 150 ommatidia. The field of view of the eye covers 170° vertically and between 120° and 210° horizontally, and the average interommatidial angle is 16°. The eyes of *L. laevigata* are most sensitive to deep blue light of 450 nm. The eye anatomy of *Nepanthia belcheri* has also been studied in some detail by electron microscopy [27]. This starfish are purported to have eyes with about 170 ommatidia. There are still controversies and dilemma whether COT starfish have eyes related anatomy, further scientific

evidence need to be extrapolated properly or tested of all this claims.

The upper convex and much darker side aboral surface is the superior integumental body wall that is of a calcareous endoskeleton. This surface is presented with numerous protruding sharp fixed spines [23, 24]. The epidermis of this integumental wall is documented to be composed of a variety of cells such as ordinary flagellated or ciliated columnar epithelial cells, neurosensory cells, mucous gland cells or goblet cells that are having finely granular contents, muriform gland cells filled with coarse spherules and the pigment granules which provide characteristic external colouration to the invertebrate. Field emission scanning electron microscope (FESEM JSM-6701F) of the integument wall revealed the presence of soft mineralized-like structures with porous concrete-like countenance suggesting primitive mineralized connective tissue matrix. The porous mineralized-like structures giving an osteoporotic-like arrangement at lower magnification. At higher magnification, the indent surface of the protuberance seems to show a spiral presentation just like the appearance on the cut surface of a tree trunk. [See Figure 4 (left) and Figure 4 (Right].

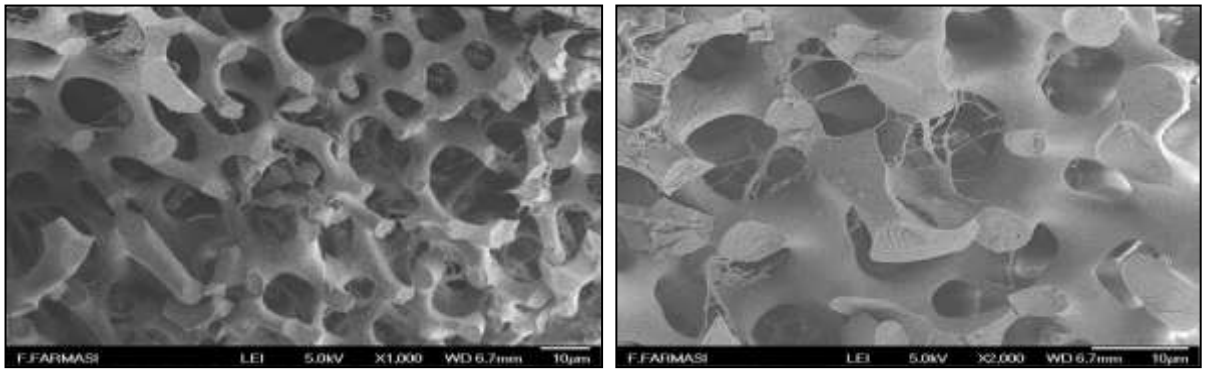

Fig-4: (left) Photomicrograph of FESEM above showed the structure of the integumental wall revealing homogenous porous mineralized plates. 1,000 x magnifications at 6.7mm WD. While Figure 4 (Right): Higher magnification AT 6.7mm WD (2,000x and 3,000x respectively) at the surface of the individual protuberance [of the integument coat] shows stages of different spiral diameters presentation. The delicate fine collagenous threads are associated within the porous mineralized structure

The COT is the second largest starfish categorized under the phylum Echinodermata that propagates sexually and asexually (Harriott *et al*., 2003). Asexual reproduction is division of the whole body by the regeneration mechanism. The sexual reproduction is done by the male and female through the release of the egg and the sperm in the water which fuses and forms the adults**.** Regeneration is a common characteristic amongst echinoderms as a form of asexual reproduction. While asexual reproduction is the most ancient mode of reproduction, it is observed in representatives of almost all marine invertebrates [30, 31]. Asexual reproduction in marine invertebrates is a very complex process that involves various mechanisms and organ systems. Because asexual reproduction is closely related to the structure of an animal, its types are as diverse as the animals themselves [32]. The variety of manifestations of this phenomenon is even greater because asexual reproduction in different species has different biological functions, such as population growth, regulation of body size, colonization of new sites, and survival under adverse conditions. The evolution of multicellular organisms has apparently passed through repeated losses and restorations of various forms of asexual reproduction [33]. Among modern groups of asexually reproducing invertebrates, COT starfish does deserve a special consideration because of their potential commercial-able added value. Experimental data on echinoderms like the COT serve as the basis for proposed mechanisms for the stimulation of asexual reproduction. Sea star and sea urchin larvae, when cultivated at the optimum temperature and with diverse food resources, is reported to undergo cloning at a higher rate. Undoubtedly, asexual reproduction plays a major role in the life activity of COT starfish and supports population size. Many problems regarding asexual reproduction in COT have still to be solved academically and scientifically. In particular, there are no studies of the cellular and molecular mechanisms of fission, or which factors (genes) determine the body of a COT to divide or undergoes fission still remains unknown lacunae.

Understanding of Crown of Thorns: Is it a predator or a waste to wealth exploitable (marine based natural products) invertebrates?

The *Acanthaster planci* Crown of Thorns (COT) starfish prevalence within the hermatypic (reef building) coral reef accounts for a large proportion of the disturbance to the coral reefs and its ecosystem due to its devastating predator ship population presence and outbreaks. The COT is not a single widespread species but cluster into four highly differentiated lineages with restricted distributions within the Pacific basin, Red Sea and the Northern and Southern Indian Ocean [34]. Cascading effects of COT outbreaks usually spread to

the entire reef ecosystem and commonly lead to increases in benthic algae, a loss of coral-feeding assemblages, and collapse of reef structural complexity that finally lead to decline in biodiversity and productivity efficiency of the reef ecosystem [35-37] The incidents of COT outbreaks are estimated to involve 90% of the coral reef destruction [38-41]. The effects of such mortality on the coral reef community are found to extend through several trophic levels [40]. The ever increasing presence and scale of devastation inflicted to coral reefs by these COT starfish outbreaks has thus served as an eye opening focused agenda for a considerable amount of scientific attention on this organism to better manage its biomass and good governance of coral reef and its ecosystem. Skeletal remains found in sediment on Great Barrier reefs show that COT have been part of the ecosystem for at least 8000 years, but the information is not sufficient to determine if there have always been outbreaks or whether only moderate densities of the starfish occurred in the past [42]. What is clear is that this disturbance to the Great Barrier reefs has never historically been so severe as to undermine its basic integrity. One theory even holds that, under natural conditions, COT outbreaks may have helped to regulate the community diversity of coral species by selectively removing the otherwise dominant *Acropora* corals, which are superior competitors in low-disturbance environments.

The basis of managing biodiversity resources globally is to ensure that these are conserved and protected subject population, hence warrant their continuous presence for sustainable demand supply chain. In addition, delineating a stock/population is important in conservation and management to avoid the overharvesting of subpopulations of a targeted species and allows defining and implementing conservation priorities. With the increased attention on sustainable development, innovation and outsourcing there is a great need for accurate, Malaysia specific evidence based assessment of COT biodiversity and listing it as a medical out source marine invertebrates. Hence, new approaches are needed which can truly show the molecular genetics and biochemical ability of a particular marine invertebrate to synthesize a metabolite or bioactive compound of tangible interest.

Natural product bioactives compound from Acansther planci Starfish

In this millennium, studies have also indicated that various starfish do possess useful pharmacological and biological characteristics and therapeutic potentials [See table 2 below]. Communities have used dried whole starfish such as *Oreaster reticulates*, *Luidia senegalensis* and *Echinaster* sp. as traditional medicine to treat asthma, bronchitis, diabetes and heart diseases in northeastern Brazil [43, 44]. Various biologically active compounds and molecules have recently been identified in starfish such as glycosylceramide, steroidal glycosides, ceramide, and cerebrosides [45, 46] Steroidal glycosides are the main metabolites of starfish and possess most toxicity [47, 48]. The metabolites of starfish could subdivide into three main groups: asterosaponins, cyclic steroidal glycosides and glycosides of polyhydroxylated steroids. Glycosides belong to the asterosaponins, and they have been reported to exhibit various biological activities, including hemolytic, cytotoxic, antifungal, antibacterial and antiviral activities [49, 50]. In another note, Luo, *et al.*, [51] reported that the starfish chemical constituent was analyzed and the toxicity of the starfish tested when it was used as mice diet. The results showed that its protein content of was 19.8 to 22.0% of its dry weight and the amino acid composition was similar to that of fish meal.

Table 2: A tabulated compilation to illustrate bioactive compounds extracts and their related activities from the global starfish taxa [communities]

	Starfish	**Geographical Origin**	**Biocompounds/ Crude Extracts in Solvents**	**Activities**	**References**
1.	Leptasterias ocholensis	Far Eastern coast	Asterosaponins & Glycosides Methanolic extract	Cytotoxic activity	Malyarenko *et al*., 2014
2.	Ctenodiscus crispatus	Sea of Okhotsk Western Pacific Ocean	Steroidal compound (polyhydroxylated steroidal derivative) Methanolic extract	Cytotoxic activity & Antitumor against Hepatocellular carcinoma & Glioblastoma cells	Tran *et al.*, [52]
3.	Ophiocoma erinaceus	Oeshn Island Persian gulf mediterranean sea in Western Asia.	Saponins Ethanolic fractions	Hemolytic and cytotoxic activity	Elaheh Amini, *et al.*, [53]
4.	Luidia	Center of Mandapam	Partially purified	Antioxidant,	Suguna,

	maculata	South East Tamil Nadu, India	compound Ethanolic extract	Antifungal & Antibacterial	Bragadeeswaran *et al*., [54]
5.	Astropecten polyacanthus	Coast of Vietnam	Asteropectinol (steroidal compound) Methanolic extract	Cytotoxic activity (potent compound against Leukemia)	Nguyen Phuong *et al.*, [55]
6.	Acanthaster planci	Indo pacific region (Australia)	Glycoprotein (toxic veneom)	Cytotoxicity	Chi-Chiu Lee *et al.*, [56]
7.	Protoreaster linckii	Center of Mandapam South East Tamil Nadu, India	Crude Compound Methanolic extract	Antimicrobial, Hemolytic, Antinociceptive & Cytotoxic activity	Suguna, Bragadeeswaran *et al*., [54]
8	Stellaster equestris	Center of Mandapam South East Tamil Nadu, India	Crude and fractioned compound-steroidal compound	Antibacterial	Suguna, Bragadeeswaran *et al.*, [54]
9	Acanthaster planci (COT)	Pulau Redang (East coast of Peninsular Malaysia)		Pepsin solubilized Collagen	Tan *et al*., [57]
10	Acanthaster planci	Pulau Redang (East coast of Peninsular Malaysia)	Homogenized crude extract	Potent Cytotoxic and apoptotic effect on Human breast cancer	Ahmed Faisal *et al.*, [58]
11	Acanthaster planci (COT)	Pulau Redang (East coast of Peninsular Malaysia)	Homogenized crude extract	Sulfated Polysaccharides glycosaminoglycans	Afiqah *et al*., [59]
12.	Astropecten indicus	Center of Mandapam South East Tamilnadu	Crude compound Methanolic extract	Antibacterial	Chamundeeswari *et al*., [60]
13.	Archaster typicus	Coast of Vietnam	Asterosaponins a. Archasterosides b. Regularosides	Cytotoxic activity against human and mouse myeloma	Kicha *et al.*, [61]
14.	Archaster typicus	Queensland, Australia	Water borne compound	Antifouling	Jana Guenther *et al.*, [62]
15	Linckia laevigata	John Brewer Reef	Water borne compound	Antifouling	Jana Guenther *et al.*, [62]
16	Fromia indica	John Brewer Reef	Water borne compound	Antifouling	Jana Guenther *et al.*, [62]
17	Cryptasterina pentagona	Kissing Point in Townsville	Water borne compound	Antifouling	Jana Guenther *et al.*, [62]
18.	Asterina pectinifera	Coast of Pohang North Gyeongsang Province, South Korea	Polysaccharides	Antitumor against colon cancer	Kyung *et al.*, [63]
19.	Culcita novaeguineae	Far east coast	Asterosaponins	Cytotoxic activity & Antitumor and chemotherapeutic agent	Guang *et al.*, [64]
20.	Asterias rubens	Coast of Norway	Coelomocytes fluid (Antimicrobial peptides)	Antibacterial	Maltseva *et al.*, [25]
21.	Anasterias minuta	Californian coast US state of California.	Steroidal Glycosides	Antifungal	Chludi *et al.*, [65]
22.	Asterina pectinifera	East coast of Korean Peninsula	Crude compound Methanolic extract	Antifungal	Choi *et al.*, [66]
23	Dermasterias imbricate	Coast of North America	Saponins, Sulfated steroidal compound	Antifungal	Bruno *et al.*, [67]

1. Marine glycosaminoglycans (GAGs) from Acansther Planci Starfish

Among the pharmacological-biological therapeutic compound is glycosaminoglycans (GAGs). Marine organisms produce a rich variety of sulphated glycosaminoglycans with characteristic variations of sugar composition and sulphation patterns determined by the species of origin. The distinct molecular structures and biophysical properties of marine glycosaminoglycans (GAGs) reflect the evolutionary adaptation and diversification of sulphated polysaccharides to complex and changing habitats. Marine GAGS are derived from organisms that have significant regenerative capacity. They may have interesting effects on the activities of many growth factors, morphogens etc. (for example FGF, HGF/SF, VEGF, GDNF) that are activated by Heparan Sulphate co-receptors. Proteoglycans (PGs) are also major components of the proteinous extracellular matrix involved in adhesion, motility, proliferation, differentiation and axon path finding (Kramer and Yost, 2003; Cattaruzza and Perris, 2005; Hacker *et al*., 2005; Holt and Dickson, 2005). PGs consist of core proteins decorated with one or more long repeating disaccharide chains called glycosaminoglycans (GAGs). GAGs are heavily sulfated molecules that interact with extracellular cytokines, growth factors and morphogens. Variations in GAG sulfation patterns can modulate their affinities for extracellular proteins (Mulloy, 2005). The status of GAG sulfation therefore plays an important role in regulating the function of PGs.

The various biochemical structural arrangement or categories of (GAGs) are majored as chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate, heparin and heparin sulfate (HS) and hyaluronan (HA) [68]. *Per se*, studies have been published that indicate breast tumour cancer nude mice have been abolished by the modified CS, and on the other hand, the tumour cell adhesion, migration, growth and invasion can be inhibited by HS mimetics with its anticancer properties.

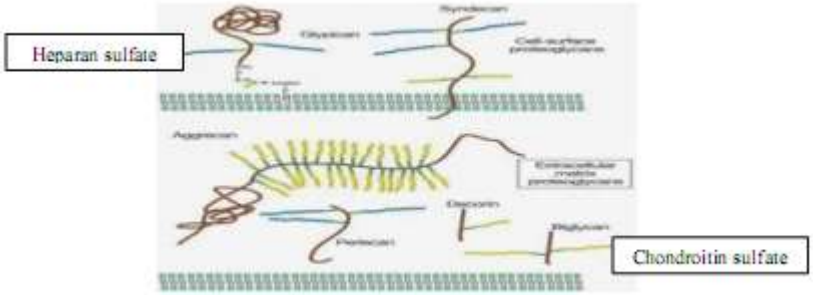

Fig-5: An illustration revealing proteoglycans core protein with one or more covalently attached glycosaminoglycan chains (heparan sulfate; chondroitin sulfate/ dermatan sulfate)

GAGs extraction from the local geo-signature *A. planci* was conducted via modification of Ledin *et al*., method [11] adapted from Staatz *et al*. The study documented that within this COT, its body's coelomic fluid contained the highest amount of total sulfated GAG, followed by body's integument, arm's internal tissue, arm's integument, body's internal tissue while the lowest amount was extracted from its regional arm's coelomic fluid. This sulfated GAGs of the local geo-signature *A. planci* influenced the invasive migration of fibroblasts during wound repair dynamics characterization [69]. This result seems to support *in-vitro* transmigration of normal adult human dermal fibroblasts from a collagen matrix into a fibrin gel, which required a cell surface mediation of chondroitin sulfate (CS) and dermatan sulfate (DS). The above publication supported evidence for the existence of GAGs in local crown of thorns starfish. Thus proteoglycans with GAGs are present and expressed in crown of thorns starfish as in other bilateria – in its basement membranes and is as part of signal transduction systems - but however large structural proteoglycans are not observed or characterized thus assumed absent. This presumably do correlates morphologically with the absence of plats of hyaline cartilage and osteonal formation within their body mass. All of this class of GAGs plays their important tangible roles in cancer progression and cell signalling. For an example, the non-sulfated HA which is synthesized at exterior surface of plasma membrane is viewed as an important participant in inflammatory condition and tissue homeostasis.

2. Antioxidant, antiviral and anticancer [cytotoxic] effects from Acansther Planci Starfish

The crude toxin extracted from Acansther Planci venom glands exhibits many diverse biological activities; mouse lethality, hemorrhagic activity, edema-formation presentation, histamine-releasing activities from mast cells [70], cardiovascular actions (Yara, *et al*., 1992, Shiromi, *et al*., 1994) and anticoagulant activity (Karasudani, *et al*., 1996). The anticoagulant action, for example, is the result of primarily derived proteins and their glycosaminoglycan side-chains (Giri & Tollefsen, 2006). Two lethal factors, plancitoxin I and II [71], two phospholipase A_2, AP-PLA_2-I and – II (Shiomi *et al*., 1998) and an anticoagulant factor plancinin, (Karasudani *et al*., 1996) have been isolated

from the spines of COT. Plancitoxin I is not only having potent hepatotoxicity [70, 71] but also have a structure that resemble to mammalian deoxyribonuclease II [71], which are implicated in DNA degradation during apoptosis [72] and/or in engulfment-mediated DNA degradation (Evans and Aguilera, 2003). Incubation of plancitoxin I with calf thymus DNA resulted in the increase of absorbance at 260 nm, demonstrating the ability of plancitoxin I to cleave DNA. In view of these data, plancitoxin I has great potential as an anti-cancer agent.

Sulfated GAGs synthesized by Golgi apparatus and altered by specific biosynthesis enzymes have shown to contribute towards different steps of tumour progression [73]. Study conducted by our research group (Mutee, *et al*. 2012) revealed that extract from the whole body mass of COT could induce significant potent cytotoxic effect on MCF-7 cells, the human breast cancer cell line. Via this research, we extrapolated that the extracted crown of thorn starfish crude exhibited significant potent cytotoxic activity on MCF-7 cells with a low half maximal inhibitory concentration (IC_{50}) value of 15.6µg/ml, $p < 0.01$. The National Cancer Institute (NCI) has reported that crude extract which exhibits cytotoxicity activity with IC_{50} value less than 20 µg/mL is considered as an active compound against cancer cells [74]. The research also demonstrated that the COTS extract induces apoptosis in MCF-7 cells. Interestingly, the apoptotic effect of the COTS extract was induced within 2 hours of treatment while apoptotic effect by the positive control drug Tamoxifen was seen only after 4 hours of treatment (Mutee, *et al*., 2012). This showed how potent the COTS extract was against breast cancer cell line.

Marine actinomycetes, isolated from the surface of marine algae and invertebrates, have received increased attention as a potential source because they produce a variety of new bioactive secondary metabolites compared to terrestrial microorganisms In pertinent to this, two new α-pyrone derivatives [an important class of lactones], violapyrones H and I, along with known violapyrones B and C were isolated from the fermentation broth of a marine actinomycete Streptomyces *sp*. The strain was derived from a crown-of-thorns starfish, Acanthaster planci. The cytotoxicity of violapyrones was assessed by sulforhodamine B (SRB) assay using human cancer cell lines. Violapyrones showed growth inhibitory activity against cancer cell lines at the concentrations less than 26.12 µg/mL [75].

In a study by Chi-Chiu Lee, *et al.,* [56], that support and scientifically also indicated that the starfish *A.planci* is a good resource for obtaining biologically active substances for antioxidant and anticancer effects, *A. planci* was extracted with 70% ethanol and lyophilized to obtained an ethanol fraction. The ethanol fraction was dissolved with water and defatted with petroleum ether to obtain a non-polar fraction. The residual solution was successively partitioned with ethyl acetate and butanol to obtain anethylacetate fraction and butanol fraction, respectively. Four fractions were used to examine the antioxidant and anticancer properties. The ethanol fraction of *A. planci* contained the highest antioxidant effects such as ABTS, DPPH, Fe2+chelating activity and reducing power when compared with four fractions. Among the four fractions, the butanol fraction was especially shown to inhibit human malignant melanoma proliferation of human malignant melanoma A375.S2 cells, which is involved in the apoptotic progression. This fraction could induce apoptosis and even necrosis in A375.S2 cells as evidenced by double staining with an Annexin V-FITC and PI assay and DNA fragmentation analysis.

Two antiviral compounds, AP-I and AP-II was also successfully purified from COTS by Shimizu, 1971. The extracted biological active compounds were found to inhibit the multiplication of influenza virus in chicken embryos. The study also showed that similar antiviral compounds present in two other species of starfish, i.e. *Asterias forbesi* and *Asterina pectinifera* [76].

3. Collagen from Acansther Planci Starfish

Living systems provide a formidable source of inspiration for the design and synthesis of new classes of materials with a potentially wide range of medical and non-medical applications [77]. Some of the most interesting and puzzling types of body walls occur among the echinoderms (starfish, sea urchins, sea cucumbers, etc.), whose body walls consist primarily of a collagenous dermis and calcite ossicles. It's true to acknowledge that the structural and mechanical properties of animals thus mostly rely on collagen organization and presence [78] one of the main protein constituents of the extracellular matrix in connective tissues and an essential component of mammalian skin, bones and tendons. Thus knowledge of the composition and structure of their collagen fibrils and interfibrillar matrix is thus important for an understanding of the physiology of these tissues. In tandem to this the local endemic *Acanthaster planci* COT is a wasted predatory marine biomass although there are publications revealing its connective tissue are enriched with collagen.

Collagen has been widely exploited as the material in food, cosmetic and biomedical industries due to its low immunogenicity and high biocompatibility [50]. At present, collagen for industrial purpose is mainly extracted from bovine and porcine origins [97]. However, the outbreak of mad cow disease and foot-and-mouth disease as well as religious restrictions have resulted in rising concerns regarding bovine and porcine collagen as a potential transmitting pathogenic vector of these diseases [50]. Mutable connective tissue are unique to echinoderms, changes

its mechanical behaviors within seconds of nervous stimulation i.e., it's able to changes between extremes of firm stiffness and flexible softness. The mutable connective tissue may be present within the entire body mass or wall, where it forms a functional system with regional muscle organization. However both the chemical basis of mutability and molecular mechanism of this phenomenon are not scientifically understood.

Local geochemical signatures COTs integument biomass and its internal tissue were inspected and were found to contained mutable collagen when examined under optical and electron microscopes [24]. Our study has successfully extracted and yield collagen from the body wall of local coral reef associated COTs [57]. Pepsin-solubilized collagen (PSC) was isolated from the inert body wall of COTs using pepsin digestion in 0.5 M acetic acid (conventional method). The electrophoretic pattern of PSC showed that it contained two main α- chains components (α1 and α2 chains), suggesting that it might be a type I collagen. While the amino acid composition analysis showed that the said PSC contained high content of glycine, proline and hydroxyproline, Fourier transform infrared spectroscopy (FTIR) investigation revealed the existence of triple helix structure of the isolated collagen. In tandem to this finding, the structure of the dorsal body wall of the starfish *Echinaster spinulosus (Asteroidea: Spinulosida),* was studied using polarized light microscopy of frozen tissues, scanning electron microscopy and histology. The collagen fibres of the body wall form a three dimensional orthogonal web. Voids in the web contain ossicles and papulae. The orthogonal web delivers dimensional stability but allows shear necessary for ray torsion. The ossicles and fibres interact to load the fibres in tension and the ossicles in compression [80].

The various studies stated above seems in agreement that the collagen molecules from echinoderm collagenous tissues are similar to mammalian fibrillar collagens in the length of their triple helices and in their quarter-staggered association to form fibrils with a characteristic D-period and gap/overlap ratio. These findings confirm and extend earlier observations on echinoderms made both by X-ray diffraction [81] and by electron microscopy [82-84].

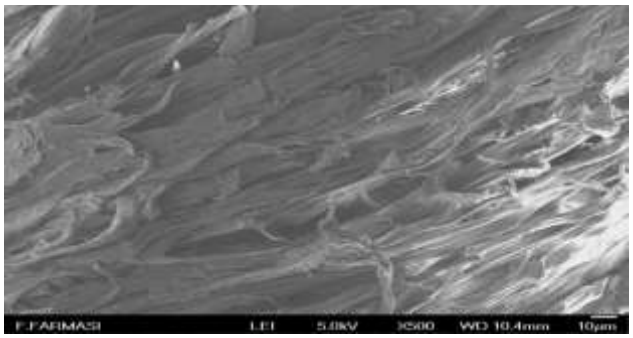

Fig-6: Field emission scanning electron microscope photomicrograph image of collagenous sheets (pepsin solubilised extracted) organisation and structure from the COT integumental wall mass

The research potential and possibilities' of pertinent to Acanthaster planci starfish

Marine ecosystem sustainability and conservative efforts with green biotechnological approaches have now been more seriously focused through intergovernmental supports and strategic action line programs. However such programs (especially in poorly and under developed country) cannot success without collaboration between academic researchers, marine/fisheries agencies and pharmaceutical industries. For example, the overall biogenetic distribution of Malaysia invertebrates and many marine fisheries are not really scientifically located and taxonomied (especially in pertinent to molecular tagging and voucher activities). The dominant pathway of any bioactive compounds biogenesis should be related to molecular elucidation and tagging especially to correlates dominancy. Hence, a detailed activities pertaining to biogenetic origins must be studied. Hence there is still a vast multi-disciplinary research lacunae's of pertinent to Acansther Planci starfish research. Among them are as stated below:

1. Molecular technology to characterize *Acanthaster planci* COT and to study its evolutionary relationship

Advances in DNA sequencing technologies have enormous potential for the medical–marine related sciences. With genome-scale data sets obtained from these new technologies, researchers are able to greatly improve understanding of evolutionary relationships, which are key to applications including marine invertebrates breeding and patho-physiology. There are literatures that seem to indicate that investigations have gave rise to broad surveys of marine life for novel natural products with useful biological properties. However, these initial efforts clearly prioritized description of unique structural chemistry rather than discovering drugs or drug leads. Molecular genetics techniques have found broad utility in modern marine ecology, and applications continue to grow. Databases

of DNA sequences now permit non-experts to identify eggs and larval stages of many marine animals that were previously mysteries. Molecular identifications of field collected organisms and tissues are used to help assess population connectivity, investigate marine food webs and identify marketed commodities.

DNA (and RNA) analyses permit estimates of genetic similarity among organisms. At the coarsest level, we can identify species. At a finer level, we can examine differences among populations within species. Levels of diversity within and between populations can also provide insights into historical population sizes and demography. Thus molecular characterization is important for identification and management of COT. For example, molecular data can be used to determine suspected tissues or products derived from COT, study genetic relationship of various COT populations in Asia Pacific region, to estimate location of their outbreak and their relationships with other factors such as ocean current and catastrophic events (e.g. flood and tsunami). Currently, several molecular techniques such as polymerase chain reaction using sequence based typing and sequence specific primer can be used for targeted screening of several regions within COT (mitochondria; 18S ribosomal RNA, control regional and cytochrome oxidase I genes) and nuclear genomes (short tandem repeats).

2. Mechano-sensory stereocilia organization

The behavior and anatomical features of marine echinoderms provide possible candidate roles on genes encoding homolog's of human deaf research. Asteroids and echinoids have several types of pedicellaria, minute jawed appendages that bite and poison ectoparasites and aid in feeding and removal of debris. The cavities of some pedicellaria have hillocks of cells having long cilia thought to be sensory. These vesicular cavities are purported to have statocysts including statoliths in their vesicular cavities that are thought to be involved in maintaining their equilibrium in relation to gravity (Hyman, 1955). These invertebrates' statocysts/statoliths have a role in geotaxis or body orientation and may have evolved into the macula of the vertebrate vestibular apparatus and eventually into the cochlea involved in hearing. It is, therefore, a plausible hypothesis that these echinoderms have mechano-sensory stereocilia that may be organized using the same proteins found in vertebrate hair cells or other cells having modified cilia and it would be interesting to test these hypotheses using the information from the genome annotation.

CONCLUSION

There is highly and ubiquitously abundant presence of bioactive compounds in inland vertebrates (especially from domestic mammals as the main representative organisms). Nonetheless, the bioactive compounds extracted from these inland vertebrates are now over exploited and thus outsourced. In recent years, many therapeutic compounds derived from marine invertebrates have been scientifically investigated and documented including on COT reviewed here. While chemistry can search, indicate and dominated the tangibility of marine natural product research in pertinent to COTs, that may resulted in the isolation and structure identification of thousands of new metabolites, it appears that increased scientific evidence database input from marine molecular biology is also much needed in order to generate an equally sound knowledge with regard to the biochemical and genetic mechanisms underlying the expression of these natural products. These are important aspects for a sustainable exploitation and commercialization of COTs, especially for health related agendas or therapeutic application.

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