1. GENERAL INTRODUCTION

Human populace has become vulnerable and affected by different types of diseases and incidentally, only less than half of all diseases can be treated efficiently. The increase in emergence of multi-resistant pathogenic bacterial strains to conventional antibiotics, especially to tuberculosis, AIDS and viral infections etc is a cause for worry. Also, emergence of new diseases like bird flu, foot and mouth disease etc are panicking the already stressed market for new medicines to combat them.

Cancer, one of the major public health burdens in developed countries, continues to represent the largest cause of mortality in the world and is the second cause of death in many developed and developing countries of the world (Nunez et al., 2006). Though many anticancer agents are used with varying degrees of success, they all have some distinct disadvantages as, such treatments may affect normal cells as well and cause serious and potentially life threatening side effects. Therefore, new anticancer treatments are continuously being sought which will prove to be more selective in inhibiting cancer cells, while being less toxic to the normal cells (Ireland et al., 1996). So, there exists a constant need to search for new therapeutic agents (Bernan et al., 1997; Strohl, 1997; Muller et al., 2000; Larsen et al., 2005).

The nature offers many benefits to the living beings including human by providing living space, protection, food and, as well as useful products with medicinal properties. Natural have been the vital source since ancient times.
and, played an important role in the treatment and prevention of human diseases. The therapeutic natural products found in terrestrial plants and microorganisms have formed the basis of early drug development (Jones et al., 2006). Natural products are still the major sources of innovative therapeutic agents for infectious and life-style diseases (Clardy and Walsh, 2004; Cragg et al., 1997). Approximately 80% of the people are mainly depending on traditional medicines for their health care as reported by the World Health Organization (WHO) (Farnsworth et al., 1985). The review on the natural products as sources of new drugs for the period 1981 to 2002 by Newman et al. (2000; 2003) provides insight into the natural resources that are being explored for the benefit of mankind.

The chemical novelty associated with natural products is unique and higher than that of any other sources. Hence, they have long been recognized as an important source of therapeutically effective medicine. The chemical novelty includes classes like terpenes, shikimates, polyketides, acetogenins, peptides and alkaloids of varying structures and a multitude of compounds of mixed biosynthesis (Wright, 1998).

The marine ecosystem, which encompasses a diverse array of fauna and flora, is represented by 34 of the 36 phyla (Faulkner, 2002) and nearly 300,000 species of plants and animals have been described from the marine realm (Jimeno, 2002; Pomponi, 1999). Marine organisms live under intense pressure for space, light and food in their environment and they, especially the sedentary organisms, are prone to predation and fouling of their surface.
So, as a mean of adaptation, they have evolved a range of defense mechanisms including behavioral, physical and chemical strategies to ensure their survival in the competitive ecosystem. They produce chemical substances, the secondary metabolites, which regulate the biology, co-existence and co-evolution of the species including ecological roles like anti-predation, mediation of spatial competition, prevention of fouling, facilitation of reproduction, protection from ultraviolet radiation and others (Sammarco and Coll, 1992; McClintock and Baker, 2001; Rittschof, 2001), without participating directly in their primary metabolic process (Torsse, 1983).

The secondary metabolites produced by marine organisms are not toxic in order to avoid auto-toxicity and are considered the natural inhibitants of the settlement of epibionts. The presence of bioactive secondary metabolites representing all the major compound classes is attributed to the ecological success of sponges in the marine environment (Ireland et al., 1988). Also, the predation is rare among the fleshy nature of soft corals due to the production of feeding deterrent diterpene metabolites (Coll et al., 1989) and secondary chemicals are used as a defense mechanism in gorgonians (Harvell et al., 1988). Some marine molluscs, notably sea hares, cuttlefish, squid and octopus release ink when attacked by predators. Though the secondary metabolites constitute a very small fraction of the total biomass of an organism (Cannell, 1998), ocean is considered as an enormous resource pool (Cragg et al., 1997) and likely to continue to be a prolific source of new natural products for many years to come (Proksch et al., 2002). In the marine environment, sponges, coelenterates and microorganisms constitute 37%,
21%, 18% of the major sources of biomedical compounds, followed by algae (9%), echinoderms (6%), tunicates (6%), molluscs (2%), bryozoans (1%), etc. (Blunt et al., 2004).

Marine natural products have many applications, including human health, food and cosmetics (Bongiorni and Pietra, 1996). Secondary metabolites from marine invertebrates have been targeted as new lead compounds in pharmaceutical, agrochemical, food and nutraceutical industries (Devries and Beart, 1995; Jack, 1998; Kerr and Kerr, 1999; Faulkner, 2000b; Blunden, 2001). The secondary metabolites produced by the marine invertebrates have been linked in some instances to the associated microbes or accumulation through food sources. For example, the antifungal cyclic peptide theopalauamide and the cytotoxic macrolide swinholide A, isolated from the sponge *Theonella swinhoei*, has been linked to the endosymbiotic filamentous and unicellular bacteria in the same sponge (Bewley *et al.*, 1996). The laurinterol acetate and laurinterol isolated from the sea hare *Aplysia kurodai* were also reported from the red alga *Laurencia intermedia* (Irie *et al.*, 1970).

The major marine groups exhibiting bioactivity are sponges, corals, ascidians, bryozoans, polychaetes, molluscs, starfish, plankton, seaweeds, fungi and bacteria. Among them, Porifera is the most studied phylum followed closely by the Cnidaria, Chromophycota, Rhodophycota, Mollusca, Chordata and Echinodermata. Invertebrates have recently become one of the hotspots of lead bioactive compounds study (Zhang *et al.*, 2005). The bioactive
metabolites isolated from the marine animals could be divided into steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, brominated compounds, nitrogen heterocyclics and nitrogen sulphur heterocyclics (Bhakuni and Rawat, 2005).

The first notable discovery of biologically active compounds from marine resources happened in early 1950s with the serendipitous isolation of the C-nucleosides, Spongouridine and Spongothyridine from the Caribbean sponge Cryptotheca crypta (Bergmann and Feeney, 1951) and the systematic investigation of the marine environment as a source of novel biologically active substances began in the mid 1970s. The number of compounds isolated from various marine organisms has virtually soared from 10,000 (MarinLit, 2001) to over 14,000 (MarinLit, 2007). Various compounds with pharmacological activities like analgesics, anti-inflammatory, antibiotics, anticoagulants, CNS (Central Nervous System) depressants etc have also been isolated.

Newman and Cragg (2004) reviewed the marine natural products and related compounds in clinical and advanced preclinical trials. A few drugs have already found a place in therapy like the antibiotic cephalotin from the marine fungus Cephalosporium acremonium and the anticancer agent arabinoside from the gorgonian Eunicella cavolini. The anticancer marine alkaloid Ecteinascidin 743 (ET 743), isolated from Ecteinascidia turbinata of Caribbean Sea, has entered into market in the name of Trabectedin (Yondelis) and has been approved in the Europe as a second line treatment
for advanced soft tissue sarcoma (Wan et al., 2007). The extremely potent venoms of predatory cone snails *Conus* sp., the conotoxins, have yielded complex mixtures of small peptides (6-40 amino acids) that have provided models for novel painkillers like Ziconotide (Cragg et al., 1999; Olivera, 2000).

The Porifera are the richest source of natural products. Sponges display a wide chemical diversity of secondary metabolites including amino acids, nucleosides, macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols (Thakur and Muller, 2004). They are known to host a large microbial community, even to an extent of 50–60% of the biomass of the sponge (Wang, 2006). Microbial associates of sponges gained significance as a source of bioactive compounds and for example, the polybrominated biphenyl ether antibiotics isolated from the sponge *Dysidea herbacea* are actually produced by the endosymbiotic cyanobacterium *Oscillatoria spongeliae* (Newman and Hill, 2006). Thakur and Muller (2004), Thomas et al. (2010) and Joseph and Sujatha (2011) have reviewed the bioactive sponge metabolites.

Rinehart et al. (1981) ranked a number of molluscs as high in the priority list of species exhibiting antimicrobial activity. The shell-less molluscs invited more attention of the researchers than the other forms. The sea hare, *Dolabella auricularia* from the Indian Ocean has been the source of 15 cytotoxic peptides, Dolastatins with remarkable *in vitro* cytotoxicity and impressive *in vivo* anti-tumour activity at low doses (Smith et al., 2001). A most potent amphotericin B, isolated from the egg mass of Spanish dancer nudibranch *Hexabranchus sanguineus*, showed potent cytotoxic activity
against L1210 murine leukemia cells and antifungal activity (Kernan et al., 1988).

Many marine plants (red, green, brown and blue-green algae) produce structurally unusual and biologically active metabolites. Seaweeds were considered to be of medicinal value in the orient as early as 3000 B.C. and the Japanese and Chinese use seaweeds in the treatment of goiter and other glandular disorders. A number of species of marine algae have been found to possess anticoagulant and antibiotic properties. Carrageenan may be useful in ulcer therapy (Kaliaperumal, 2003) and the alginates are found to prolong the “rate of activity” of certain drugs (Mathieson, 1969). Many beneficial properties of the dietary algae include anti-oxidant, anti-viral, anti-inflammatory, anti-bacterial, anti-tumor and anti-wrinkle (Montano, 2008).

Though the marine environment harbours microbes, typically ranging from $10^3$ to $10^6$ per milliliter with as many as $10^9$ per milliliter in marine sediments (Austin, 1988), Cragg and Newman (2005) opined that less than 1 % of bacterial and 5 % of fungal species were known till 2005. Bacterial associations are quite common among marine invertebrates. The striking similarities between invertebrate metabolites and the known microbial metabolites have raised a question on the real origin of invertebrate metabolites. Some bioactive compounds isolated from the invertebrates originate from symbiotic microorganisms. Hence, marine microbes received much attention over a period of time (Blunt et al., 2007). Symplostatin 1, a close structural analogue of dolastatin 10 isolated from the marine mollusc
*Dolabella auricularia*, currently in phase II clinical trials, was found to be a metabolite of the blue-green alga *Symploca hydnoides* (Harrigan *et al.*, 1998). The reviews by Laatsch (2006), Lam (2006) and Bhatnagar and Kim (2010) highlighted the enormous potential of marine microbial metabolites.

Ascidians are the prolific producers of bioactive substances and are a notable source of nitrogen-bearing secondary metabolites with a wide range of biological activities (Seleghim *et al.*, 2007). Didemnin-B from the Caribbean tunicate *Trididemnum solidum* was the first marine compound to enter human cancer clinical trial as a purified natural product (Carte, 1996). Ecteinascidin-743, a tetrahydroisoquinoline alkaloid from the colonial tunicate *Ecteinascidia turbinata* from the Caribbean and Mediterranean seas possess very potent activity against a broad spectrum of tumor types in animal models (Rinehart, 2000). Dehydrodidemnin B, isolated from a Mediterranean tunicate *Aplidium albicans*, was in Phase II studies in the United States and Europe to determine its anticancer properties (Erba *et al.*, 2002). New potent cytotoxic lamellarin alkaloids were isolated from Indian ascidian *Didemnum obscurum* (Reddy *et al.*, 2005). Halocynthiaxanthin and fucoxanthinol from *Halocynthia roretzi* induced apoptosis in human leukemia breast colon and other cancer cell lines (Konishi *et al.*, 2006).

Bryozoans, called moss animals or ectoprocts, are tiny colonial animals that occur in both freshwater and seawater, mostly in marine. They are abundant in temperate-tropical waters that are not too turbid. They grow on a hard substrate and feed on suspended food. More than 5000 bryozoan
species have been documented so far worldwide and in India, about 200 species have been reported (Venkataraman and Wafar, 2005). Though the bryozoan diversity is rich, only 1% of natural products were characterized from bryozoans (Blunt et al., 2003). Bryozoans are rich in bioactive secondary metabolites and such metabolites have ecological significance (review by Sharp et al., 2007). Christophersen (1985) reviewed the secondary metabolites from bryozoans. Bryostatins, isolated from the marine bryozoan Bugula neritina are the best known examples of bryozoan secondary metabolites with potent cytotoxic activities (Pettit et al., 1982; Pettit, 1991). The other bioactive secondary metabolites from marine bryozoans include alkaloids, sterols, as well as heteratom-containing compounds with antitumour potential against many cancer cell lines (Tian et al., 2011).

Indian marine natural product research gained momentum only in the late seventies (Nittala et al., 2006). India has a few ethno histories of marine substances used in traditional medicines. In Indian Siddha medicine, corals are used for treating cough, nervous disorders and tuberculosis (Formulary of Siddha medicines, 1972). Sponges are used to treat goiters in Indian traditional medicine (Radhika et al., 2008).

Gulf of Mannar, located along the southeastern part of Tamil Nadu, is a marine biosphere reserve extending from Rameswaram to Kanyakumari. In Gulf of Mannar, 117 species of corals, 641 species of crustaceans, 631 species of molluscs, 441 species of finfishes, and 147 species of seaweeds along with seasonally migrating marine mammals like whales, dolphins,
porpoises and turtles have been reported (Kumaraguru, 2006). The diversity is unique in this ecosystem and the animals are expected to have evolved adaptive measures to survive this highly competitive habitat.

The objective of the present study is to investigate the bioactive potential of the invertebrates found along the Gulf of Mannar coastal waters especially,

- to study the antibacterial activity of extracts of the 29 selected invertebrates against human bacterial pathogens;
- to study the pharmacological (analgesic, anti-inflammatory, CNS stimulant, antipyretic and anti-ulcer) potential of active extract;
- to isolate the potent compound by chromatographic techniques and characterize the same by spectrometry techniques;
- to study the anticancer property of the active extract and the possible mode of cytotoxicity to cancer cell lines.