General introduction
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The potential of natural products has been recognized since antiquity. They continue to contribute a great deal to modern industries by providing a wide range of chemicals such as antibiotics, cardiac drug and insecticides. These were discovered from living organisms and are most vital to modern life. The explosive growth in modern biology has created a new awareness of the unlimited biotechnical potential natural products such as plants, microbes and animals (Banerji, 1992). The diversity of life in the terrestrial environment is extraordinary and it consists of 36 phyla of organisms whereas the world oceans are represented by 34 phyla. The oceans cover more than 70% of the earth’s surface and contain more than 30,000 described species of plants and animals (Jimeno, 2002 and Pomponi, 1999). The diversity of species is extraordinarily rich on coral reefs and there are around 1,000 species per m² in some areas (Pomponi, 1999). The ocean provides a huge resource bank to the discovery of novel compounds (Cragg et al., 1997). Many compounds are diverse, novel and bioactive have been isolated from marine organisms. Natural Products with secondary or non-primary metabolites are produced by living organisms, have been exploited for a variety of purposes including use as food, fragrances, pigments, insecticides, herbicides and medicines. The seas provided a suitable site for the early evolution of all life. Ever since plants and animals developed structures and mechanisms that enabled them to survive on land, terrestrial and aquatic plants have been exposed to different abiotic and biotic selective pressure.
Modern natural products research began approximately 40 years ago with the study of organisms from intertidal and shallow subtidal environments, where samples are easily accessible by wading and snorkeling. In the 1960s, the use of SCUBA diving as a scientific tool enabled researchers to begin looking at shallow water subtidal organisms to depths of approximately 40m. The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products (Carte, 1996). From 1969 to 1999, approximately 300 patents on active marine natural products were issued. From humble beginnings, the number of compounds isolated from various marine organisms has virtually soared and now exceeds 10,000 (MarinLit, 2001). Since then, over 14,000 different natural products from marine organisms have been described (MarinLit, 2003) and hundreds of patents describing new bioactive marine natural products have been filed (Kerr and Kerr, 1999). Approximately 10 to 15 different marine natural products are currently in clinical trials mostly in the areas of cancer, pain or inflammatory diseases (Proksch et al., 2002).

To date, researchers have isolated thousands of natural products from marine organisms such as 25% from algae, 33% from sponges, 18% from coelenterates (sea whips, sea fans and soft corals) and 24% from representatives of other invertebrate phyla such as ascidians (also called tunicates), opisthobranch molluscs (nudibranches, sea hares etc), echinoderms (starfish, sea cucumbers etc) and bryozoans (moss animals) (De Varries and Bearts, 1995; Rinehart et al., 1988; Yamada et al., 2000; Rinehart, 2000; Oliveira et al., 1985 and Potts and Faulkner, 1992). A simplistic analysis reveals the search of "Drug from the Sea" is in progress resulting in 10% increase of new compounds per year. An increasing number of novel marine metabolites are reported in the literature, indicating that the marine environment is likely to continue to be a prolific
source of new natural products for many years to come (Proksch et al., 2002). Researchers are concentrating their efforts on slow-moving or sessile invertebrate phyla that have soft bodies and lack of spines or a shell that require a chemical defense mechanism (Faulkner, 1995).

The success of marine natural products as drugs and the fact that the ethnological and biological resources from which they originate remain underexploited continues to provide a significant impetus to the drug discovery. Since the first antibiotic from a marine bacterium was described in 1966 (Burgess et al., 1999), the number of new compounds has increased constantly during the years. Even though only a few compounds from marine organisms might be interesting for the pharmaceutical market today (Faulkner, 2000), some bacterial species are already used as bio-control and are added to aquaculture stocks. Most secondary metabolites from marine bacteria found so far were isolated from *Streptomyces* sp and *Alteromonas* sp (Wagner et al., 2002). Symbiotic and associated microorganisms are often proposed as the true producers of natural products isolated from marine invertebrates. Numerous examples exist, in which structurally related compounds have been reported from taxonomically distinct invertebrates and cultured microorganisms alike. Several recent examples of metabolites isolated from sponges and ascidians are structurally related to microbial products. These findings support the hypothesis that many invertebrate-derived products are of microbial origin (Moore, 1999).

Analyses of microbial communities have been hindered by our inability to cultivate most of the organisms within a sample. Estimates of bacterial recoverability from environmental samples range from 0.01 to 12.5% of the existing community (Fenical, 1993; Amann et al., 1995; Sobecky et al., 1998 and Ward et al., 1990). A
number of studies have investigated the effects of various environmental parameters (e.g., starvation, heating, freezing chlorine, pH etc.) on the recoverability of microorganisms. The simple act of taking microorganisms from their natural environmental stresses subjects them to unnatural growth conditions. These injured cells are commonly unable to produce colonies on media used for their enumeration. Therefore, recovery of microorganisms from environmental samples is likely reduced as a result of potential stress and injury. In an effort to minimize injury and stress and thus improve detection of these microorganisms, workers have advocated the exogenous addition of various supplements, most often catalase or sodium pyruvate to culture media (Olson et al., 2000). Most of these studies focused on the recovery of specific organisms rather than improved overall recovery from an environmental sample for antibacterial activity. Microorganisms examined include *Escherichia coli* as an enteric indicator species (Calabrese and Bissonnette, 1990), numerous microaerophilic organisms such as *Campylobacter fetus* (Hoffman, et al., 1979), *Treponema pallidum* (Steiner et al., 1984), *Clostridium perfringens* (Harmon and Kautter, 1976) and *Spirillum volutans* (Padgett et al., 1982).

Molluscs which are widely distributed throughout the world have many representatives in the marine and estuarine eco-systems. Among the marine organisms the molluscs are one of the most successful forms of animal life and they have conquered almost every habitat and exist in all the oceans. The majority of natural product studies of the mollusca have been in the class of Gastropoda. Many studies on bioactive compounds from molluscs exhibiting antibacterial, antitumour, anti-leukemia and antiviral activities have been reported worldwide (Carte, 1996; Myers et al., 1993; Hochlowski and Faulkner, 1983; Ireland et al., 1993; Pettit et al., Faulkner and Ghiselin,
1983; Roesener and Scheuer, 1986; Faulkner, 1988; Morris et al., 1990 and Schmitz et al., 1993 and Fontana et al., 2001. Interest in the chemistry of molluscs has been based on the finding that many concentrate metabolites from the highly specialized diets and incorporate them into their own defensive strategies (Faulkner, 1988). One of the most novel and well-studied macromolecular compound, antineoplastic was isolated from the mollusc *Mercenaria mercenaria* and the familiar name of the compound is ‘mercenene’ (Li et al., 1974).

Insects, weeds and phytopathogenic microorganisms cause great damage to agriculture. An estimated one-third of a typical crop is lost due to pests and diseases and these are not systematically controlled (Melnikov, 1971). The judicious application of herbicides has been an integral part of agriculture (Piementel, 1986). Much of the increase in agricultural productivity over the past half century has been due to the control of these pests with synthetic chemical pesticides (SCPs) (Duke, 1993). Crop protection chemicals continue to be the major tool for protecting food and fiber crop from damaging pests. In 1990, the world market value of pesticides totaled nearly 23 million dollar, 28% of which was for insecticides. In the hunt for new agro-chemical agents the plants, animals and microorganisms of the marine environment with their wide range of chemical diversity prove to be an unexplored resource. To date, research focused on isolating insecticide prototype leads from marine origin has resulted in the report of about 40 active compounds. Insecticidal compounds were isolated from red algae (Watanabe et al., 1989), corals (Grode et al., 1983) marine annelids (Okaichi and Hashimoto, 1962) and many important herbicides from marine algae (Fenical, 1981).
In the pharmacology of natural products, a wide range of useful drugs have been isolated from plants and animals. Although it is impossible to compile a full inventory, these drugs include analgesics, anti-inflammatory, antibiotics, anticoagulants, CNS depressants, hormones and vitamins. The marine environment is an exceptional reservoir of bioactive natural products having potential biomedical applications, many of which differ from those of terrestrial organisms in both chemical structures and peculiarities of biological actions (Faulkner, 2003) and sea has immense biomedical potential which can be exploited not only as a source of drugs for treatment of disease but also for new and novel structures with useful biological activity. 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 covaloni*. Various marine toxins like tetradotoxin and saxitoxin are not only important pharmacological tools but can be used in terminal cases of cancer for relief of pain (Munro *et al*, 1987; Abraham, 1977 and Narahashi, 1988).

India has a long maritime history. The peninsula is surrounded by the Arabian sea, Bay of Bengal and Indian Ocean. The vast coastline of the mainland and islands is about 7500 Kms and its territory includes 1256 islands. The area of Exclusive Economic Zone (EEZ) of the country is 2.02 million sq. Km. The vast coastal and offshore environment supports a wide variety of marine ecosystem rich in species diversity and multifarious economic development activities. The sea has tremendous influence on the physical and meteorological conditions of the country. Appreciating the importance of the subject, Government of India established Department of Ocean Development in 1981 with an aim of creating a deeper understanding of the oceanic regime of the northern and central Indian Ocean and also development of technology and technological aids for harnessing of resources and understanding of various physical, chemical and biological processes.
The Gulf of Mannar, which is having 21 islands extending to about 628 hectare, is unique for its biodiversity. A variety of coral reef habitats allow the islands to support complex biological communities. The Tuticorin region of the Gulf of Mannar forms the southern part of the Marine National Park. There are numerous species of organisms in tropical waters, particularly in coral reefs of Tuticorin coast. The diversity of species in a coral reef is comparable to even greater than that in a rainforest. Many bottom dwelling invertebrates such as sponges, soft corals and tunicates have no value as food and hence have not been considered as economically important resources. However, chemical and pharmaceutical studies in the past three decades have revealed that these unpalatable organisms are important sources of biologically active substances which have potential to be developed into new drugs and other useful products. Because of high diversity of species and hence of high competition for survival, coral reef organisms, particularly lower invertebrates which lack the physical means of defense, have evolved a variety of unique toxic compounds as their defense strategy. Many of these compounds have no terrestrial counterparts in the chemical structures as well as biological activities. These facts, together with a high incidence of bioactive compounds, have made coral reef organisms especially attractive targets of research.

Tuticorin sector of Gulf of Mannar is having rich molluscan diversity including commercially important cephalopods, bivalves and gastropods. The common gastropods available along Tuticorin coast are Chicoreus ramosus, Chicoreus virgineus, Pleuroloca trapezium, Babylonia spirata, Hemifusus pugilinus, Xancus pyrum, Drupa sp., Lambis lambis, Strombus sp., Conus sp., Trochus sp., Oliva sp., Cypraea sp., Tonna dolium, Rapana sp., Murex tribulus, Cymatium sp., Tibia sp., Umbonium sp., Cymbium sp., Harpa sp., Phalium sp., Natica sp., Turbo sp and Cerithidea sp. (Murugan and
Edward, 2000). *Drupa margariticola* and *Trochus tentorium* are commonest gastropods inhabiting the reefs and may be seen adhering the corals rocks in large numbers. Studies on bioactive compound from gastropods is limited and mostly concentrated on cones toxins. Among the Muricids, *Chichoreus ramosus* extract were screened for antibacterial activity (Kagoo and Ayyakkannu, 1992). Potent acetylcoline inhibitors are reported for the mollusc, *Babylonia japonica* (Ireland *et al.*, 1993).

The immune defense of molluscs is non-specific and lacks inducible immunoglobulins, but the responses against microbial organisms are based on the both cellular (phagocytosis, encapsulation, respiratory burst, etc.) and humoral (lectins, agglutinins, lysosomal enzymes, antimicrobial factors, etc.) activities (Canesi *et al.*, 2002 and Chu, 1998). A variety of antimicrobial factors including chlorinated acetylenes (Walker and Faulkner, 1981), terpenes (Ireland and Faulkner, 1978), indole derivatives (Benkendorff *et al.*, 2001a), glycerol derivatives (Gustafson and Anderson, 1985), macrolides (Matsunaga *et al.*, 1986), pigments (Sanduja *et al.*, 1985), lysozymes (Nilsen *et al.*, 1999) glycoproteins (Yamazaki, 1993), proteins (Kamiya *et al.*, 1989) and peptides (Charlet *et al.*, 1996 and Mitta *et al.*, 1999) have been isolated from molluscs. Although some of the low molecular weight compounds might be diet derived (Gustafson and Andersoen, 1985, Okuda and Scheuer, 1985) or produced by microbial symbionts (Barbieri *et al.*, 1997 and Seiderer *et al.*, 1987). These reports suggest that molluscs are a rich source for discovering novel lead compounds for the development of new types of antibiotics for pharmaceutical use.

The aim of the present work is to investigate extensively the bioactive potential of the molluscs; *Drupa margariticola* and *Trochus tentorium* which are abundantly associated with coral reef of the Tuticorin coastal water. With a preface effort, the crude
extracts and partial purified fractions of the selected molluscs were tested against human and fish bacterial pathogens and were examined for the presence of insecticidal and herbicidal properties. The pharmacological (analgesic, anti-inflammatory activity and CNS depressant) potential of the 100% Acetone column-purified fractions was assessed. To improve the microbial recoverability, media supplement like sodium pyruvate was used in order to increase the percentage of antagonistic strains from soft corals, *Sinularia* sp. and *Lobophytum* sp. The isolation of the surface associated bacteria from scleractinian corals such as, *Acropora formosa*, *A. nobilis*, *Favia palida*, *Favites abdita*, *Turbinaria mesenterina* and *T. peltata* and our initial testing of those bacteria for ability to inhibit the growth of human and fish pathogenic bacteria *in vitro* was also experimented. The precise antibacterial activity compound was isolated by Column chromatography and TLC and purified through HPTLC and HPLC, final structure characterized by Nuclear Magnetic Resonance (NMR) technique, Infrared Resonance (IR) and mass (MS) spectrometry.