Ethnopharmacological Properties of Muricidae Gastropods

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Muricidae Gastropods

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Abstract

Marine organisms account for approximately half of all global biodiversity, with the mollusca family containing the second highest number of species, which includes snails and bivalves, after the aforementioned mollusca. The marine environment is highly competitive, hostile, and aggressive, prompting molluscs and their associated microorganisms to produce specific and potent bioactive compounds in order to protect themselves and ensure their survival. Most important bioactive compounds have been well characterised, and some have already reached clinical trials or been approved for use as therapeutic agents and supplements, derived from marine mollusc extracts. This review focuses on some of the pharmacological properties obtained from marine molluscs with special reference to muricidae gastropod as well as the challenges associated with bioprospecting for valuable drugs from marine mollusc sources.

Chapter - 1

Introduction

Nature is a primary source of effective medicinal agents, and folk medicine has been the basis for the development of a large number of drugs. Plasters, ointments, and wound dressings based on animal fat, for instance, have been in use for millennia. Along the history, humans have searched in nature how to obtain resources for their basic needs (Cragg and Newman, 2001). Many years of observation and experimentation have provided medical knowledge in the use of natural products (Alves and Rosa, 2013). Around 60% of commercially available drugs are based on bioactive compounds extracted from natural sources (Cragg and Newman, 2013). A great number of these natural products have come to the market from the scientific study of remedies traditionally used by various cultures around the world (Cragg and Newman, 2013). Zootherapy is defined as healing human diseases using animals or animal-derived products (Costa-Neto, 1999). Nowadays, the use of animals with medicinal properties is a common practice worldwide. In China, more than 1500 animals are used as medicine; in India 15 to 20% of the Ayurvedic medicine is based on animal-derived substances. In Brazil, 326 animal species are recorded with medicinal purposes, whereas 584 medicinal animal species are reported in Latin America (Alves and Rosa, 2005).

India is one of the 12 mega-biodiversity countries in the world. It comprises of 25 hot spots, which constitute the richest habitat for the highly endangered eco-regions of the world. Perhaps, among the Asian countries, India is the only country that has a long record of coastal and marine biodiversity, dating back at least two centuries. It has a coastline of about 8000 km with an exclusive economic zone of 2.02 m/sq km adjoining the continental regions and offshore islands. Moreover, it comprises a wide range of coastal ecosystems such as estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches, and coral reefs. But today, the sudden environmental changes, over exploitation and habitat loss of the coastal and marine biodiversity components remain the major reasons behind the day-to-day species loss.

The Mollusca is a phylum of marine invertebrates that are of particular interest as a source of new potential drugs leads. Molluscs encompass 7% of living animals on the planet making them the second largest animal phylum with estimated 100–200 thousand species, of which more than 52 thousand have been described and named (Benkendorff, 2013). The phylum Mollusca also includes eight different classes: Gastropoda, Bivalvia, Scaphopoda, Cephalopoda, Polyplacophora, Monoplacophora, Caudofoveata and Solenogastres (Benkendorff, 2015). which illustrates a significant evolutionary divergence over the past 500 million years. The molluscs have been involved in the everyday life of humans in many cultures throughout history, providing a source of shells, food, dyes and medicine. In addition to the use of molluscs for magical-religious purposes. The shelled molluscs are considered as healthy food and some species could be value-added as functional or medicinal foods, as they are still an important element in a variety of traditional natural medicines (Léo Neto *et al*., 2012). The marine invertebrates, molluscs lack acquired immunity and essentially depend on their innate immunity and bioactive compounds to protect against microbial pathogens and heal wounds in the microbially-rich marine environment (Dang *et al*., 2015).

The molluscs have been a significant focus in the search for biologically active secondary metabolites with more than 1,145 natural products isolated from molluscan species in the last three decades (Benkendorff, 2010). The derived compounds from molluscan have been clinically tested and approved by the Food and drug admiration, especially drug of ziconotide from cone shells for the treatment of lymphoma and Hodfkin's disease (Mayer *et al*., 2010).

The gastropod (soft bodied unsegmented animals) belongs to the large and diverse phylum Molluscs. It includes a variety of animals well-known for their decorative shells or as seafood. For illustration, it includes the tiny snails, clams and abalone as well as the larger animals such as squid, cuttlefish and octopus. They occupy a variety of habitats ranging from mountain forests, freshwater and the sea benthos at a depth of more than 10 km. Their size ranges from less than 1 mm to more than 15 m (for example the giant squid) and their population density may have exceeded even up to 40,000/m2 in some areas. In the tropical marine environment, the molluscs seem to occupy all the tropic levels ranging from primary consumers to top carnivores. They have been categorized under different taxonomic groups such as, mussels, oysters, clams, pearl-oysters, window-pane oysters, ark-shells, whelks, chanks, cowries, squids and cuttlefishes.

Many natural products are sourced from the marine environment due to its phenomenal biodiversity. Marine invertebrates (Porifera, Echinodermata, Cnidarian, Mollusca, Arthropoda) have to date provided a substantial diversity of natural products, including terpenes, alkaloids, aliphatic hydrocarbons, steroids, carbohydrates, amino acids and peptides (Leal *et al*., 2012). These marine-derived natural products have an extensive array of therapeutic properties, including anticoagulant, antimicrobial, wound healing and immune modulating, antioxidant, anticancer, anti-inflammatory, antihypertensive, and other medicinal properties (Perdicalis *et al*., 2013). A number of marine natural products have provided important leads for drug development and many are now used in the formulation of novel drugs (Leal *et al*., 2012). For example, Ziconotide, first isolated from the cone snail *Conus magus* venom effectively blocks N-type voltage gated calcium channels and is effective for the treatment of chronic pain. The drug has now been Food and Drug Administration (FDA) approved and has been commercialised under the name of Prialt ® (Svenson, 2013).

Chapter - 2

Bioactive Compounds from Marine Molluscs

In recent days, the rich diversity of marine fauna includes sponges, molluscs, bryozoans, ascidians, etc., which provides a great opportunity for the discovery of new bioactive substances (Thakur and Miller, 2004). The number of potential compounds isolated from the marine realm has nearly tripled, but this figure does not exceed 10,000, with hundreds of compounds yet to be discovered (Torres *et al*., 2002). The bioactive components provide a variety of metabolites, some of which can be used for drug development (Chellaram and Prem Anand 2010). For instance, the Tyrinian purple, an ancient dye pigment, is the first natural product of marine origin reported in literature. Similarly, the mantle cavity of muricid gastropods (rock snails) is known for producing mucus, which possesses an immense ability to defend the developing larvae against microbial infection (Simmons *et al*., 2005). Therefore, such marine natural products (secondary metabolites) have to be investigated predominantly for their antimicrobial, cytotoxic, antitumour, antiviral, anti-leukemic, and anti-inflammatory properties.

Some marine gastropods and bivalves have been of great interest to natural products chemists, yielding a diversity of chemical classes and several drug leads currently in clinical trials. Molluscs also feature prominently in a broad range of traditional natural medicines, although the active ingredients in the taxa involved are typically unknown. Overall secondary metabolites have only been investigated from a tiny proportion (<1%) of molluscan species. At the class level, the number of species subject to chemical studies mirrors species richness and our relative knowledge of the biology of different taxa. The majority of molluscan natural products research is focused within one of the major groups of gastropods, the opisthobranchs (a subgroup of Heterobranchia), which are primarily comprised of soft-bodied marine Molluscs. Conversely, most molluscan medicines are derived from shelled gastropods and bivalves. The complete disregard for several minor classes of Molluscs is unjustified based on their evolutionary history and unique life styles, which may have led to novel pathways for secondary metabolism. The polyplacophora, in particular, have been identified as worthy of future investigation given their use in traditional South African medicines and their abundance in littoral ecosystems. As bioactive compounds are not always constitutively expressed in Molluscs, future research should be targeted towards biosynthetic organs and inducible defence reactions for specific medicinal applications. Given the lack of an acquired immune system, the use of bioactive secondary metabolites is likely to be ubiquitous throughout the Mollusca and broadening the search field may uncover interesting novel chemistry.

Chapter - 3

Economic Importance of the Marine Molluscs

Molluscs are one of the most delicious and protein rich foods among sea foods. Moreover, they serve as cheap food, raw materials for cottage industries, two Jagadis (2005). The shell remains discovered in pre-vedic Mohanjedaro, Harappa, Amri, Na, Nundara, and Rupar human habitations reveal evidence of a long-standing association between man and mollusk in India. The evidence includes not only the cowries (Cyprea sp) and chank (*Xancus* sp) but also their products. They are also exploited in preparing soups in many countries, such as Japan, Korea, and China. Commercially, they are in high demand in shell craft production, such as lampshades, curios, fashion accessories like buttons, mother of pearl flakes from powdered scrap, and formulation of lacquers and shampoos, etc. Widespread usage has often led to indiscriminate exploitation, and it is important to utilise this resource in a sustainable manner. To overcome the demand for gastropods, seed production of many commercially important gastropods has been successfully developed in hatcheries for the culture activity in the country (Figure.1).

Many types of molluscs are eaten worldwide, either cooked or raw, due to their rich nutritional value. Furthermore, they are used as pearls, cowry, and accessory materials, for tools like household dishes, cooking pots, and utensils such as spoons, cutlery, scoops, spatulas, etc. Some of them are destructive and caused ecological damage. Some serve as intermediate hosts for human parasites. Some can cause damage to crops. Mollusc meat is known to be highly nutritious and salutary owing to its high content of essential amino acids, proteins, fatty acids, vitamins, and minerals. In addition, some of the bioactive compounds, including antiviral, antimicrobial, antiprotozoal, antifungal, antihelminthic, and anticancer products, are produced by molluscs. The largest edible snail is the African land snail, *Achatina achatina* mostly consumed by African people. Due to their aphrodisiac effect, molluscs were a very prominent dish during the Roman Empire. Some mollusc species include zinc and essential amino acids, which keep up body energy and boost sex drive as an aphrodisiac, strengthen the immune system and make bones stronger. Pearls are highly valued bivalve products that contain nacreous deposits that contain 82–86% calcium carbonate (aragonite crystals), 10–14% organic substance conchiolin, and 2-4% water. Cowry is a marine snail from the genus Cypraea, dwelling mostly in the coastal waters of the Indian and Pacific oceans and used instead of money. Money cowry (*Cypraea moneta*), a 2.5cm yellow species, has served as currency in Africa.

A commercially important edible marine gastropod is observed to inhabit the marine coastal waters in sandy benthic zones. Whelks, as they are commonly known, are easily caught and landed in shrimp trawlers in Kerala and Tamil Nadu. They are traditionally exploited for the shell-craft industries of Tamil Nadu. However, in the early 90s, the whelk fisheries gained significance, because of the demand for exporting their meat to certain Asian countries like, Japan and Singapore. Their shells are mainly collected for their operculum, which are used in lime making, incense sticks, cosmetic production, perfumes, pharmaceuticals, and beautiful items such as curtains, pen stands, mementos, key chains, and other novelties. Handicraft items made from their shells are widely sold in almost all cities and tourist centres in India. At present, there is a good market for them not only in India but also in western countries.

The deadly venoms of some cone shells (Conidae) are today being used to help victims of strokes and heart disease, and to produce a revolutionary new drug for chronic pain control called Ziconotide. An extract from the hard clam, *Mercenaria mercenaria* L. is a strong growth inhibitor of cancers in mice. The drug is called Mercenine. Ground and processed oyster shells are used as calcium supplements both for humans and animals. Paolin, a drug made of abalone juice, is an effective inhibitor of penicillin-resistant strains of bacteria such as Staphylococcus aureus, Streptococcus pyogenes, and Salmonella typhus. Oyster juice has been found to have antiviral effects and may be made into a drug eventually (Lakshimi, 2001). The venom of cone snails used for hunting their prey can be dangerous, even lethal for humans. The venom of cone snails used for hunting their prey can be dangerous, even lethal for humans. The venom is a neurotoxin and is being studied for use in medicine.

A scarce number of reports deal with molluscan natural products' effectivity against their peculiar viral infections, but, on the other hand, a large number of human viral pathogens have been found susceptible to molluscan metabolites, reflecting a lack of tailored antiviral response in their innate immune system (Dang *et al*., 2015). Secondary metabolites isolated from no less than eight gastropods (*Haliotis laevigata*, *Haliotis rubra*, *Haliotis rufescens*, *Littorina littorea*, *Buccinulum corneum*, *Tegula gallina*, *Rapana venosa*, and *Buccinum undatum*) and nine bivalves (*Mercenaria mercenaria*, *Mya arenaria, R. philippinarum, Cerastoderma edule, Mytilus galloprovincialis, Crenomytilus grayanus,* Crassostrea virginica, Crassostrea gigas, and Ostrea edulis) have been described as potent against many, generally human, viruses (Benkendorff, & Speck, 2011). An extraordinarily large number (roughly 100,000 species) of mollusks remain untested for potential antiviral efficacy (Dang *et al*., 2015). Proteins and glycoproteins are the most abundant compounds found in molluscan extracts active against viruses (Dang *et al*., 2015). To quote some examples, kelletinin A (B. corneum) has been found active against Human T-cell leukemia virus type 1, hemocyanin (R. venosa) against respiratory syncytial viruses, Herpes simplex virus (HSV)-1 and -2, and Epstein–Barr virus (EBV) (Genova-Kalou *et al*., 2008; Nesterova *et al*., 2011), mytilin (M. galloprovincialis) against white spot syndrome virus (Dupuy *et al*., 2004), defensin (M. galloprovincialis) against human immunodeficiency virus type 1 (HIV-1) (Roch *et al*., 2004), and lectin (C. grayanus) against HIV (Luk'yanov *et al*., 2007). Though their exact mechanism of action is hitherto unknown, apparently, they counteract invading viruses either by directly inactivating them and preventing their binding to or entrance into target cells or by inhibiting their replication and transcription (Dang *et al*., 2015). Moreover, molluscan species are generally active against an extensive array of viral families.

The absence of a well-defined adaptive immune system compels mollusks to adapt their innate immune system accordingly in order to cope with invading pathogens (Hooper *et al*., 2007; Liu *et al*., 2009; Tiscar & Mosca, 2004). A key constituent of the innate immune response in marine mollusks is represented by antimicrobial peptides. The mass of these antimicrobial peptides is (sea hare) (Yamazaki, Ohye, Kisugi, & Kamiya, 1990), and in the body wall of another sea hare, *Dolabella auricularia* (Iijima *et al*., 2003). Similarly, antibacterial and antifungal peptides have been purified from the blood of M. edulis (Charlet *et al*., 1996), while an antimicrobial peptide from the mRNA and plasma of M. galloprovincialis (Mitta, Vandenbulcke, Hubert, & Roch, 1999). According to an estimate, the number of antimicrobial peptides isolated from the two main groups of molluscs, gastropods and bivalves, is 69 (Li, Parisi, Parrinello, Cammarata, & Roch, 2011). Application of molluscs other than diet

1. Products and metabolites obtained from molluscs as medicines
2. Cosmetic industry uses molluscs for products
3. Pearls as precious jewelry obtained from molluscs
4. Ancient money cowry and accessories from mollusc shells

5. Byssus from molluscs processed for valuable thread and fabric
6. Cleaning the water for better environment via molluscs
7. Qualified dyes, ink and glues obtained from molluscs
8. Inspiration from molluscs for surgical studies

Fig 1: Commerical importance of molluscs

Chapter - 4

Molecular Phylogenetic Context for the Muricidae

The muricid gastropods are among the best-known and most widespread neogastropods. They are esteemed by shell collectors for their striking range of shell shape and sculpture. In classical antiquity muricids were used by the Phoenicians, Greeks and Romans as the source of Tyrian purple dye, extracted from precursors in the hypobranchial gland, which performs a defensive function in the snails and their egg capsules (Westley *et al*., 2006). The family Muricidae comprises about 1600 extant described species, inhabiting worldwide tropical to polar seas, and ranging from the intertidal zone down to more than 3000 m depth. Typically, muricids are generalist predators, mainly of barnacles and bivalves (Taylor *et al*., 1980), but some are highly specialized, such as Coralliophila and related species, which are ecto- and endoparasites of anthozoan cnidarians. Because of their predatory behaviour, muricids play an important role in structuring marine benthic communities (Harding *et al*., 2007). Some muricids are economically important as pests of commercial bivalve shellfish, including the oyster drills Ocinebrina and Urosalpinx (Buhle and Ruesink, 2009), while others are themselves consumed as food (e.g. Murex, Concholepas, Trunculariopsis, Bolinus, Chicoreus). Despite this variety of interests for muricids, the phylogeny of the family remains poorly known, and its classification is still largely based on general shell and radular resemblances (Table.1).

Chapter - 5

Classifications of the Muricidae

Table 1: Classifications of the muricidae

Families	Subfamilies
Muricidae	Muricinae Ocenebrinae Trophoninae Typhinae Rapaninae
Muricidae	Muricinae Purpurinae
Muricidae	Muricinae Rapaninae Drupinae
Muricidae	Muricinae Aspellinae Ocenebrinae Trophoninae Typhinae
Coralliophilidae Muricidae	Muricinae Thaidinae Coralliophilinae, Muricopsinae Ocenebrinae Trophoninae Typhinae Tripterotyphinae Ergalataxinae Kuroda,
Coralliophilidae Muricidae	Muricopsinae Ocenebrinae Trophoninae Typhinae Tripterotyphinae Ergalataxinae Rapaninae Haustrinae Coralliophilinae

The fossil history of the Muricinae and Ocenebrinae made an early attempt to draw evolutionary trees based on resemblance of shells and radular types, dividing these taxonomic groups into three evolutionary ‘clans’, and suggesting that the Muricinae might not be monophyletic. The first formal phylogenetic analysis of Muricidae/Muricoidea was based on morphological characters, including features of the head-foot, radula, pallial complex, alimentary and reproductive systems of four muricid species (Harasewych, 1984).

Chapter - 6

Historical use of Muricidae Gastropods Natural Products in Ethnomedicine

Ethnomedicine is a study or comparison of traditional medicine based on bioactive compounds in plants and animals and practised by various ethnic groups, especially those with little access to western medicine, e.g., indigenous people. Throughout history, mollusks have been an important part of many cultures' ethnomedicine. For example, they were used to treat inflammation in Medieval Eastern Mediterranean, Ancient Greco-Roman, and European communities and are still used by Zimbabwean, Indian, and Latin American people. There is also evidence that different parts of molluscs were used as remedies to treat a range of inflammatory diseases. For example, the opercula from Strombidae and Muricidae were used in eastern Mediterranean medicine to treat a range of conditions, including skin diseases, stomach ulceration, and arthritis, by smelling the aromatic substances in the smoke of the slowly burned opercula (Benkendorff *et al*., 2015). The gastropod shells are the most commonly used part to treat inflammation, with more than 50 preparations documented as useful anti-inflammatory remedies. Other parts of gastropods which are often used as anti-inflammatory in TCM include flesh, with flesh from eight different species being used. Muricidae species are sent from India to European nations for use in makeup, scents, and drug development (Edward and Ayyakannu, 1992). One kg of the muricidae operculum costs Rs.900 in the home-grown market in south India. The operculum was also explicitly used to treat female conception problems such as period irregularities, atresia of the uterine cervix, and various uterine infections, as well as to remove the placenta after labor. The operculum are still regarded as important fixings in the perfume industry. The employment of these aromas containing operculum has a solid association with marriage, and only ladies or wedded ladies can apply them.

Molluscs have been used in ethnomedicine by many traditional cultures to treat different aspects of inflammatory conditions. 104 different anti-inflammatory preparations from a variety of molluscan species were tested, of which 70 were from the well-documented Traditional Chinese Medicine

(TCM). This traditional use of Molluscs has driven the testing for inflammatory activity in extracts from some species in the phylum Mollusca, with 20 in vitro studies, 40 in vivo animal studies, and 14 human clinical trials performed to substantiate the anti-inflammatory and wound healing activity of Molluscs. Some of the studies have led to the approval of mollusc-derived products to be used as over-the-counter (OTC) nutraceuticals, like Lyprinol® and BiolaneTM, from the New Zealand green-lipped mussel Perna canaliculus. Natural products, including anti-inflammatory agents, provide an important lead for the development of pharmaceuticals. Only a small proportion of the molluscan traditional medicines have been tested to confirm their anti-inflammatory activity, and most screening studies have tested crude extracts from molluscs without any chemical characterization.

Marine organisms have been shown to possess bioactive compounds with anticancer activity, and bioactive peptides from a host of marine molluscs as well as their synthetic analogues have been discovered and are under investigation for their anticancer properties. An example is Kulokekahilide-2, a cyclic depsipeptide derived from the marine mollusc *Philinopsis speciosa*, which has been found to be cytotoxic in vitro against the leukaemia cell line P388, the human ovarian cancer cell line SKOV-3, the melanoma cell line MDA-MB-435 and A-10 embryonic rat thoracic aorta medial layer myoblast cells with IC50 values ranging from 4.2 to 59.1 nM (Kang *et al*., 2018). Another peptide is Keenamide A (Fig. 1), isolated from the marine mollusc *Pleurobranchus forskalii* (Wesson *et al*., 1996). Keenamide A, a cyclic hexapeptide, exhibited significant in vitro activity against the lymphocytic leukaemia cells (P-388) and human adenocarcinoma cells (A-549) with an IC50 of 2.5 g/ml as well as the human colorectal adenocarcinoma tumour cells (HT-29) with an IC50 of 5.0 g/ml (Wesson *et al*., 1996). *Meretrix meretrix* yielded a 15 kDa linear peptide known as Mere15 (Liu *et al*., 2012; Wang *et al*., 2013, Pangestuti and Kim 2017). In vitro, Mere15 inhibited the growth of K562 leukaemia cells in vitro (IC5038.2 g/ml) and the growth of A549 cell xenograft in nude mice in vivo (IC5031.8 g/ml). The cytotoxicity of Mere 15 is due to the peptide's ability to induce apoptosis, cause microtubule disassembly, and arrest the cell cycle (Liu *et al*., 2012, Wang *et al*., 2013, Pangestuti and Kim 2017). The peptides SCHP9 (LeuProGlyPro) and SCHP10 (AspTyrValPro) were isolated from *Sinonovacula constricta* hydrolysates and shown to inhibit the growth of the prostate cancer cell lines DU145 and PC3 in a dose- and time-dependent manner (Huang *et al*., 2017).

Kanagasababathy *et al*., 2011; Shukla, 2019). A saccharothrixmicine peptide with antimicrobial activity against Candida albicans and

Xanthomonas spp. was isolated from the marine mollusc Anadara broughtoniin association with *Saccharothrix espanaensis* AN 113 (Shukla, 2019, Mitta *et al*., 2000). A cysteine-rich peptide, myticin, has been isolated from the Mediterranean mussel (*Mytilus galloprovencialis*). Shukla, 2019, Mitta *et al*., 2000). Myticin has potent antibacterial activity against both gram-negative and positive bacteria (Shukla, 2019, Shukla *et al*., 2000). Peptides with antifungal properties have also been isolated from the blue mussel (Mytilus edulis) (Mitta *et al*., 2000; Charlet *et al*., 1996) and from Mytilus coruscus (Sun *et al*., 2014).

Some bioactive antioxidant peptides have been isolated. -tocopherol can also scavenge hydroxyl radicals (IC5028 M) and superoxide radicals (IC5078.97 M) (Shukla, 2019, Qian *et al*., 2008). Furthermore, peptides obtained by hydrolysis with proteolytic enzymes of the viscera and body of the clam (Meretrix casta) resulted in significant DPPH radical scavenging activities and reducing power (measured as the ability of the hydrolysate to reduce iron III) (Pangestuti and Kim 2017). A peptide hydrolysate derived from blue mussel (Mytilus edulis) and designated BMCH has also been reported to exhibit antioxidant activities as indicated by DPPH radical scavenging (IC500.35 mg/ml) and ABTS+ radical scavenging activity (117M TE/Mg sample) (Mitta *et al*., 2000). BMCH also showed a protective effect against H2O2-mediated oxidative injury in human umbilical vein endothelial cells (HUVECs) by increasing cellular antioxidant capacities, including increasing levels of catalase, glutathione (GSH), glutathione peroxidase, and superoxide dismutase, thus indicating that BMCH may be useful in protecting against endothelial dysfunction and related diseases (Oh *et al*., 2019).

The use of muricidae traditional medicines to treat women's problems implies possible effects on steroidogenesis. Steroid hormone production is critical for maintaining the menstrual cycle, for the development and production of mature oocytes, and for establishing and maintaining pregnancy (Leung and Adashi 2004). In women, the steroidogenic pathway is expressed within the granulosa cells of the ovary and in uterine and placental cells. The human JAr Chorio placental cancer cell line and primary-derived granulosa cells from healthy women express functional steroidogenic pathways in vitro. They respond to human chorionic gonadotrophin (hCG) in vitro by upregulating steroidogenesis and increasing the levels of progesterone and estradiol secreted into the cell culture medium (Edwards *et al*., 2014; Edwards, 2012). The effects of D. orbita extracts and compounds on the production of progesterone and estradiol in granulosa cells, with and without hCG (Edwards, 2012). In addition, several synthetic isatin and indirubin compounds were tested for effects on basal progesterone production in granulosa and JAr cells.

Chapter - 7

Bioactivity of Muricidae Extracts and Compounds

The pharmaceutical industry is growing rapidly and continuously. In spite of the large number of drugs produced each year, the demand for the discovery of new drugs is imperative. The motivation to discover new drugs includes the advent of new diseases and infections such as Middle East respiratory syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS); an increase in the occurrence of old diseases such as hemolytic fevers and tuberculosis; the occurrence of new challenges in old diseases such as AIDS; the growing number of drug-resistant infectious diseases as well as the highly toxic nature of some currently used drugs.

The extracts from a number of Muricidae species have been tested for antimicrobial activity against a range of human and marine pathogens, as well as marine bacteria isolated from biofilms. Ramasamy and Murugan (2005) undertook a major screening project with a wide range of molluscan extracts against 40 biofilm-forming bacteria isolated from Indian marine substrates. Included in this screening programme were whole body extracts from eight Muricidae species, as well as egg mass extracts from four species, digestive gland extracts from two, and the operculum from one. With the exception of digestive gland extracts, all showed inhibitory activity against some of the marine bacterial isolates in at least one of the solvent fractions (Armas *et al*., 2010). The egg mass extracts were the most effective, inhibiting all 40 of the test bacteria. The nonpolar solvent extracts used in this study are likely to contain significant amounts of fatty acids and sterols that could contribute to the observed bioactivity. With known antibacterial activity, the whole-body extracts from a number of Muricidae appear to be dominated by polyunsaturated fatty acids (Armas *et al*., 2010, Zarai *et al*., 2011). Conversely, no antibacterial activity was found in a mixture of saturated fatty acids and sterols modelled on the lipid extract composition from the egg mass of several Muricidae species (Benkendorff *et al*., 2005). Some muricid species are known to contain multiple choline esters, predominantly from the hypobranchial gland (Roseghini *et al*., 1996). Similarities in the pharmacological properties of the gland extracts and choline esters have been demonstrated in vitro using the frog rectus assay. These compounds elicit neuromuscular blocking action but do not bind to muscarinic acetylcholine receptors, correlating with affinity for nicotinic cholinergic ligand-gated ion channels (Keyl and Whittaker, 1958). Keyl and Whittaker (1958) were able to measure depolarization in the endplate region of rat gracilis muscle at concentrations of 3 mg/kg, whereby murexine may be bound to nicotinic acetylcholine receptors. The concentration-dependent effect of murexine on twitch reduction has also been tested in vitro on cat, dog, and rabbit gastro-cnemius (Erspamer and Glasser 1957). Muricidae extracts have demonstrated wound healing properties and anti-inflammatory activity in addition to their antimicrobial properties. Muricidae produce a suite of brominated indoles with anti-inflammatory, anti-cancer, and steroidogenic activity, as well as choline esters with muscle-relaxing and pain-relieving properties. These compounds are traditionally used for wound healing, stomach pain, and menstrual problems.

The Muricidae are predatory marine gastropods that are historically known for the production of the ancient dye Tyrian purple. The main dye pigment, 6,-dibromoindigotin, was the first marine natural product reported in the literature and has attracted substantial scientific interest (Baker 1974, Benkendorff 2000, Cooksey 2001, Baker and Duke 1976, Westley *et al*., 2006). Tyrian purple is produced by a series of enzymatic, oxidative, and photochemical reactions (Cooksey 2001, Westley *et al*., 2006) from a choline ester precursor salt of tyrindoxyl sulfBenkendorff. and its intermediate brominated indole precursors are also found in muricid egg masses (Benkendorff 2000, Westley *et al*., 2006, Benkendorff 2000, Palma *et al*., 1999), where they are thought to chemically defend the developing larvae against microbial infection. Preliminary studies on egg mass extracts suggest these intermediate precursors possess anticancer activity (Westley *et al*., 2006; Vine *et al*., 2007), in addition to the previously described antibacterial activities (Benkendorff 2000). These unique bioactive secondary metabolites are in the chemical class of indole alkaloids, from which 60% of all medicines are derived.

The brominated intermediate precursor, 6-bromo-2-methylthioindolin-3-one (tyrindoleninone, Figure 1(c)), has been identified as a useful anticancer drug lead with apparent specificity toward lymphoma cells (LC50 4 M) compared with freshly isolated human mononuclear cells (LC50 195 M) (Vine *et al*., 2007). However, synthesis of this substance has been problematic due to the instability and rapid oxidation of the precursors and final product. Nevertheless, a number of related substituted isatin derivatives have been synthesised and tested for anticancer activity against a range of human cancer cell lines (Vine *et al*., 2007). The synthesised isatins include 6-bromoisatin (Figure 1(f)), a natural oxidative by-product of Tyrian purple synthesis, which is also found in Muricidae egg masses (Benkendorff, 2000; Benkendorff *et al*., 2001).

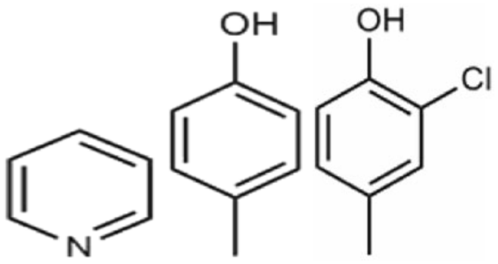
Dye secretion and extracts from the hypobranchial and reproductive glands of male muricids have also been shown to contain the structural isomer of Tyrian purple, 6,-dibromoindirubin (Figure 1(g)). Bioassay-guided fractionation of hypobranchial gland secretions has identified this isomer as a potent protein kinase inhibitor (Meijer *et al*., 2003; Leclerc *et al*., 2001). By selectively targeting glycogen synthase kinase-3 (GSK-3), these indirubin compounds effectively inhibit cell proliferation. Indirubin derivatives have also been shown to inhibit Stat3 signaling, inducing apoptosis in human breast and prostate cancer cells. They also suppress tumour necrosis factor (TNF)-induced NF-B activation in human leukaemia and lung adenocarcinoma cells and significantly block proliferation in lung carcinoma, stomach carcinoma, and fibrosarcoma cell lines (Kim *et al*., 2007).

The homoeopathic cure Murex species are also referred to as "the Murex remedy." It is also derived from the dye secretions of the Muricidae and has been in use since the 1800s (Dunhum 1864). The Murex remedy is listed on the Homeopathic Material Medica for use against a range of "women's problems", including uterine and breast cancer (Murex 1999, Cazalet 2009). In most cases, there is little scientific data available to support the biological activity of homoeopathic remedies, and few have been tested for safety and effectiveness using rigorous methodologies (Straws 2000, Pitari 2007).

Chapter - 8

Pharmacological Properties of Muricidae Opercula Oil

The chemical investigation of gastropods has led to the isolation of a wide variety of bioactive primary and secondary metabolites (Voultsiadou, 2010). These may be synthesised by the molluscs themselves, accumulated from dietary sources, or produced by symbionts. More than 1145 compounds have been isolated from marine molluscs, including peptides, sterols, terpenes, polypropionate, macrolides, fatty acid derivatives, nitrogenous compounds, and alkaloids. An additional 145 molluscan compounds have since been documented in annual Marine Natural Product Reports covering the years 2014–2018 (Carroll *et al.*, 2020). The below mentioned biomolecules are present in the operculum of muricidae species. which is used to treat a variety of diseases Pyridine Para cresol Chloro-methylpheno



Applications

Mix a few drops of opercula oil with the other incense ingredients. Inhalation of incense smoke to cure stomach pain, liver ailments, epilepsy and irregular menses

Rheumatism or arthritis, stomach ulcers, skin diseases, teeth problems, eye and ear diseases, tumors, epilepsy, paralysis, purgatives, and treatment of uterine diseases.

Muricidae gastropod operculum was used to treat a wide range of conditions, including swollen spleen, depression, rheumatism or arthritis, stomach ulcers, skin diseases such as boils, warts, and tumors, tooth problems, eye disease, hearing loss, epilepsy, and paralysis (Mchugh, 2013).The operculum extracts were also reported to be useful as purgatives and laxatives

(Voultsiadou, 2010). Periodical use of the current allopathic drugs produces many side effects in patients. Hence, there is a need for an alternative therapeutic approach (Figure 2). Rheumatoid arthritis is a chronic inflammatory disease of the joints characterised by a specific plan of destruction for bones and joints and associated with pain. Its risk factors include genetic, hormonal, environmental, nutritional, and socio-economic factors, age and sex, ethnicity, smoking; and infections (Kobayashi *et al*., 2008). Many analgesics and anti-inflammatory drugs, including steroids are used to suppress these symptoms. Some commercial drugs, DMARDs, etanercept, infliximab, and adalimumab, anti-CD20 therapy (rituximab) and abatacept are often required to inhibit or halt the underlying immune process (Coussens and Werb, 20002).

The burning of operculum is said to produce a strong odour, the scent of which is described as being similar to that of castoreum, the musk derived from the scent gland of mature beavers (Castor spp., producing an animalic or leathery note (Meyenhof and Sobhy 1932). The Arabic term "azfar al-tibb" means "sweet hoof18," and it is used to describe the operculum of Chicoreus ramosus (Murex infatus) from Bahrain, which was reported to be the best for fumigation (Meyenhof and Sobhy 1932, Levey and Maswaih 1961).There is historical evidence for the use of opercula across a number of diverse cultural groups separated by time and geographical location. Neogastropod opercula are still used today in the incense manufacturing industries of India (Mchugh 2013, Lipton, 2013) and as an important ingredient in traditional Sudanese perfumery, particularly as smoke baths for new brides or married women (Nawata 1997). In contemporary trade, the opercula of the Muricidae species Chicoreus ramosus are exported from India to European countries for use in cosmetics, perfumes, and medicines (Edward 1992).

To reconcile the long and culturally diverse practise of utilising opercula in traditional medicine and religious ceremony, further research on the bioactive secondary metabolites and volatile compounds from opercula is warranted. In this study, Dicathais orbita was used as a model species of Muricidae because it has been subject to extensive chemical and biological studies. Several methods were used to prepare the operculum based on historical and contemporary methods for processing the operculum for use in the production of incense and traditional medicines. Comparative analyses were undertaken on smoke extracts from the opercula of Chicoreus ramosus as a representative species historically employed as an incense and perfume ingredient from antiquity to the present day (Table 2 and Table 3).

Aqueous and alcoholic solvation is another common process for preparing opercula in traditional medicine. Water or alcohol based decoctions were used in traditional Chinese medicine for treating tumours and ulcers, expelling toxins from the body and relieving infammatory conditions, whereas vinegar and alcohol mixtures were used in Central European, Middle Eastern and ancient Greek medicine for arthritis, dental diseases, epilepsy, and removal of the placenta. The specific methods used to prepare the opercula medicine sold in Jewish pharmacies during the mediaeval 11th–14th centuries are unknown, but they may have included extraction in water or alcohol.Therefore, we also examined polar (methanol) extracts of D. orbita opercula

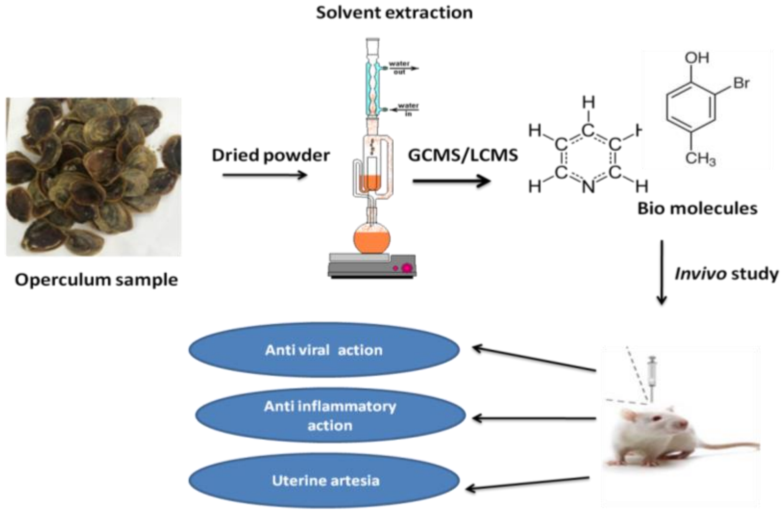
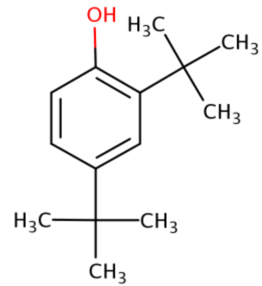
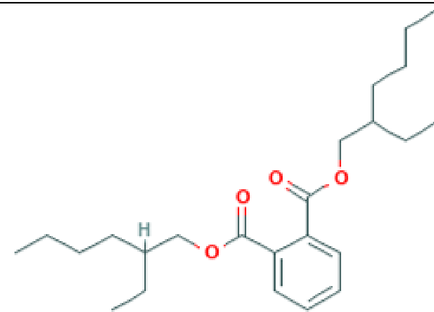
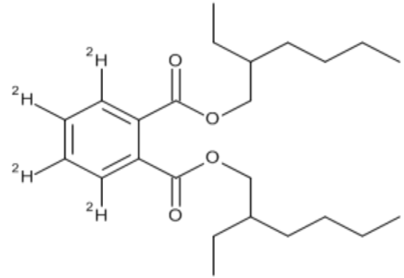
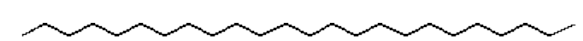
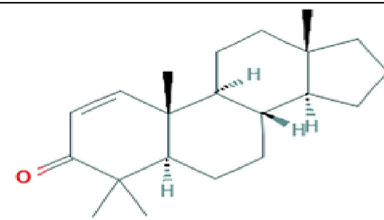
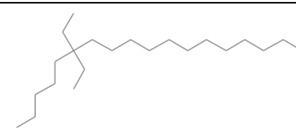


Fig 2: Diagrammatic Methodology for drug development from muricidae gastropod

Table 2: Natural compounds available in muricidae gastropod

Name of the compound	Molecular formula	Chemical structure	Molecular weight	Peak area
2,4-Di-tert-butylphenol	C14H22O		206	149.05
Bis(2-ethylhexyl) phthalate	C24H38O4		340	57.10

Bis(2-ethylhexyl) ester			842	
Eicosyl isopropyl ether	C23H48O		340	57.10
Hexacontane	C60H122		842	
Tetracosane	C24H50		338	57.10
Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)	C21H32O		184	55.05
6,6-Diethylhoctadecane	C22H46		310	57.10

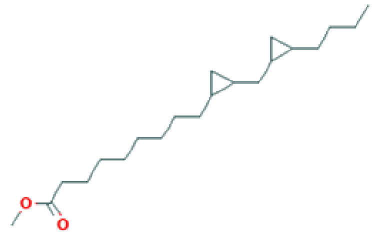
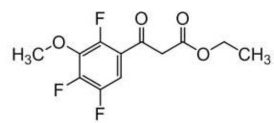
(1,1Bicyclopropyl)-2-Octanoic acid,	C21H38O2		322	73.05
2,6-Lutidine 3,5-dichloro-4-dodecylthio-	C19H31Cl2NS		375	207.00
3-Methoxy-2,4,5-trifluorobenzoic acid	C27H43F3O3		472	207.00

Table 3: Ancient medicinal uses of operculum of muricidae gastropod

Part of snail	Source species	Preparation	Pharmaceutical properties (Treatment)
Operculum	*Bolinus brandaris* *Thais haemastoma*	Crushed and mixed with oil and vinegar	Hearing loss, swollen spleen, depression, menstrual Cycle, abnormalities, after labour for placenta removal
Operculum	*Murex anguliferus*	Smell the aromatic substance or Smoke produced while placing Diseases the operculum on slowly Burning charcoal	Rheumatism or Arthritis Stomach problem (wounds in stomach), skin Diseases, teeth problems eye and ear diseases Tumours, epilepsy, paralysis purgative, treatment of diseases of the uterus.
Operculum	*Chicoreus ramosus*	Ustulate the shell, Ground into powder and apply externally	Skin diseases; wounds in the stomach; arthritis; eye and ear diseases; treatment of uterus; diseases,
Operculum	*Rapana* spp.	Decoct the operculum (10–20 g) and ingest; Ustulate the shell, ground into Powder and apply externally.	Swelling and ulcer on the body surface
Operculum	*Chicoreus virgineus*	Smell the aromatic substance or smoke produced while placing the operculum on slowly burning charcoal	Rheumatism or arthritis; stomach problem (wounds in stomach); skin diseases; eye and ear diseases; tumors; treatment of uterus diseases

Chapter - 9

Nutritional Contribution of Muricidae Gastropods to Human Diets

Human beings have eaten molluscs for thousands of years, and nowadays, molluscs are considered a common food consumed by millions of people worldwide, particularly in European countries (Murphy *et al*., 2001; Jess and Marks, 1998). Additionally, the cephalopods, which include the cuttlefishes, squids, and octopus, are generally operated for food fish in most parts of the world. In most countries, oysters and gastropods are used in dishes and eaten as an aperitif meal. Mollusk meat (escargot) is not only tasty but also has several advantages over others: quite a low lipid rate and calorie values versus rich mineral, essential amino acid, and fatty acid content, especially polyunsaturated fatty acids (PUFA). With higher omega-3 fatty acid content, snail meat is found to be a factor affecting a longer lifespan and a lower cancer rate in most countries. Calcium, magnesium, zinc, copper, manganese, cobalt, and iodine are the predominant minerals in molluscs' flesh (Yildrim *et al*., 2004, Ekin and Bashan 2010, Ekin *et al*., 2011). The nutritional resources of the oceans are being explored and exploited to meet the nutritional challenges of an increasing world population. Gastropods have assumed commercial importance as a nutritional resource by virtue of their high-quality meat and vast demand in the regional and international markets, particularly in the Mediterranean and Asian countries (Mason *et al*., 2014). In India, gastropods that were considered bycatch or were discarded earlier, now form an exclusive gastropod fishery. Muricid gastropods (family: Muricidae) are gaining therapeutic acceptance as there is potential to derive high-value protein significance (Naegel and Cooksey, 2002). The detailed nutritional profiling of this muricid gastropod species is still nonexistent. Although a preliminary attempt has been made (Ramesh and Ayyakkannu, 1992), the study was limited to the proximate composition analysis without detailing the quality of the lipid, protein, and mineral content in the candidate species. The increasing demand for gastropods in the international market as an important commercial fishery commodity has occurred only during the last few decades as a recognition of their nutritional qualities. Recently, there has been a tremendous increase in the market value of the meat of muricidae and it has evolved as a potential candidate for sea farming (Nasution and Roberts, 2004). Ramesh and Ayyakkannu (1992) reported the crude lipid content in the edible part of the microcode gastropod of C. ramosus as 2 mg/100 g-1.

Nutritional composition of muricidae gastropod operculum

S. No	Nutritional composition	Percentage
1.	Moisture	7.88 ± 0.12
2.	Crude Protein	6.31 ± 0.10
3.	Crude Carbohydrate	3.40 ± 0.03
4.	Lipid	2.15 ± 0.02
5.	pH	6.76 ±0.02
6.	Ash	3.05 ± 0.01

The food industry is one of the most important manufacturing sectors, and its significance in catering to the dietary needs and economy has been further enhanced with the advent of the contemporary functional food and nutraceutical market. The abundance and diversity possessed by marine organisms make them an important constituent in the supply chain of this flourishing manufacturing sector. Apart from being an important source of food, marine organisms are continuously creating curiosity in the scientific community because of their potential role in the functional food and nutraceutical industries. Molluscs, being the second most abundant group of animals and having demonstrated medicinal value, are probably the most noteworthy group of marine organisms in this regard. Many molluscan species find use in traditional medication in different parts of the world due to the presence of a diverse array of active ingredients. Such traditional applications of mollusks have been augmented by many scientific reports relating to their biological activities. Moreover, they are relatively easy to catch, and their commercial breeding and farming are rampant in many parts of the world. With a focus on the antiviral, anti-inflammation, and antimicrobial potency of molluscs and products thereof, this review has gathered the bulk of information in relation to biologically active molluscan products and their potential application in the functional food and nutraceutical industries. Consumption of molluscs as part of an everyday diet can also provide certain health benefits as they are rich in vital nutrients and active secondary metabolites, as well as have the ability to enhance immune response. Furthermore, since normal cooking practises do not significantly alter the composition of these organisms, they are bound to retain some, if not all, of their bioactivities after cooking. The consumption of many molluscs in some Asian societies exclusively for their health-promoting efficacy suggests that certain bioactivities associated with them can be retained even after the action of digestive enzymes. Actually, this fact has been successively supported by a number of reports that point toward the efficacy of molluscan consumption in fighting various diseases. Indirect, but scientifically sound, evidence in this regard can be found in the large number of reports on the effectiveness of orally administered molluscan preparations. Though the phylum Mollusca has enjoyed momentous attention from the scientific community and a number of commercial nutraceuticals of molluscan origin have been marketed, the number of compounds isolated from molluscs is not in line with the number of species therein. The promising potential of molluscs and products thereof in relation to antiviral, anti-inflammatory, antimicrobial, and other activities calls for more exploration and validation in order to fully utilise this enormous source of food and drugs.

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