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Antimicrobial secondary metabolites from marine gastropod egg capsules and egg masses

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ABSTRACT

Marine organisms have attracted special attention in the last three decades for their ability to produce interesting pharmacological active compounds. Even though all marine organisms have the potential to produce antimicrobial secondary metabolites, the gastropod has the vital sources of secondary metabolites particularly their egg capsule which has the promising antimicrobial secondary metabolites. In the present review, we intend to focus on marine secondary metabolites from marine gastropod egg capsule. The following compounds *i.e.* Kabiramid C, Aplysianin E, Aplysianin A, Thisaplysianin E and Tyrian purple have been documented in egg capsule of various gastropod and most of the antimicrobial secondary metabolites have not been isolated from the egg capsule because of the odious, and complex chemical structure. Stability of the compounds is unknown.

1. Introduction

Prosobranches are the most conspicuous egg capsule producing gastropods. The prosobranches deposit their egg capsules on a variety of living and nonliving hard substrata like shells and rocks, small stones and blades of seaweeds, sandy and mud flats. It also deposited their egg capsule in other gastropod egg capsule. *Rapana rapiformis* selected the egg capsules of *Chicoreus virgineus* for depositing its capsules.

These capsules have many putative functionalities including protection against desiccation and predation, osmotic stress, and UV radiation, as effective shock absorber. The early stage shell-less embryos of the gastropod egg capsule were both found to suffer high mortality outside their leathery egg capsules. However, some embryos can be reared outside the capsule in non-sterile seawater after they have developed shells.

During the past 30 years, a core group of marine natural products (MNPs) chemists from several countries, in collaboration with both academic pharmacologists and the pharmaceutical industry, have reported a very large number of novel metabolites with useful and sometimes sensational pharmacological properties. However, MNPs are exhibiting high antimicrobial activities. The gastropod egg capsule possesses vast number of active secondary metabolites which have much antimicrobial activity due to the function of protecting embryos inside the capsule. Most of the studies concerning antimicrobial activity include egg masses or capsules, this study was aimed to document the active MNPs from gastropod egg capsule.

2. Secondary metabolites from marine gastropod egg capsule

The first attempt to locate antimicrobial activity in marine organisms was initiated around the 1950s[1]. The anticarcinogenic toxin, palytoxin, has been found in high concentrations in the eggs of zoanthids in the genus, Palythoa[2]. Furthermore, there is evidence that the requirement for antimicrobial agents in some molluskan egg masses diminishes as the embryos develop. Sea hare eggs contain a large quantity of an antibacterial glycoprotein, but its physiological function has not yet been clarified. The

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free-swimming larva is initially a trochophore, which later develops a velum-like fold and thin shell and becomes the characteristic molluscan veliger larva in an egg capsule[3].

Only very few molluscs are appeared to provide parental care. The majority of the egg masses are left unprotected in the hostile marine environment. But, certainly they might possess some sort of defensive mechanism to protect the developing embryos. The egg capsule contents of *Nucella lapillus* were shown to be free from bacterial contamination[4]. The absence of bacteria in the internal matrix is a unique phenomenon in a complex and hostile environment and the investigation of the responsible substance may lead to novel antibacterial substance.

2.1. Kabiramide C

Lipophilic extract of egg masses of an unidentified nudibranch collected at Kabira Bay in Ishigaki-jima Island of the Ryukyus showed considerable antifungal activity and the antifungal agent from the Kabira collection a major active compound, named kabiramide C, has been assigned a novel macrolide structure. Kabiramide C showed marked antifungal activity[5].

Antitumour and antifungal macrocolides have been isolated from the egg ribbon of nudibranch *Hexabranchus sanguineus*[5]. The gelatinous egg masses of the sea hare Aplysia Juliana have been shown to lose antimicrobial activity during embryonic development[6].

Trisoxazole macrolides are cytotoxic and antifungal metabolites initially isolated from the egg-ribbons of the *Hexabranchus nudibranch* and later found in other marine invertebrates. They possess a characteristic macrolide portion, in which three contiguous oxazole units are integrated, and a side-chain with an N-methyl-vinylformamide terminus. The planar structures of the first members of this group, ulapualides and kabiramide C, were determined by interpretation of spectral data in conjunction with chemical degradation. Following these studies, the structures of approximately 35 congeners have been reported, including mycalolides from a marine sponge *Mycale sp.* The absolute stereochemistry of mycalolides was determined by chemical methods. Trisoxazole macrolides depolymerize F-actin and form a 1:1 complex with G-actin, thereby exhibiting potent toxicity toward eukaryotic cells. X-ray crystallography established the mode of binding of some of the members to G-actin and their absolute stereochemistry[7]. Recently studies have developed fluorescent kabiramides: new probes to quantify actin in vitro and in vivo[8]. Biomolecular mimicry in the actin cytoskeleton: mechanisms underlying the cytotoxicity of ha studied in kabiramide C and related macrolides[9].

Three trisoxazole macrolides possessing a 30-R, β-enone moiety, including the known kabiramide G (1) and the new kabiramides J (2) and K (3), were isolated from the sponge *Pachastrissa nux*, along with the previously reported Kabiramides B (4), C (5), and D (6). To date, the enone moiety has been found to associate solely with the trisoxazole macrolides from *Pachastrissa nux*. All of the isolated macrolides showed moderate to strong antimalarial and cytotoxic activities, except for 1, which possessed only potent cytotoxicity[10].

A hybrid compound consisting of Aplyronine A and Mycalolide B was synthesized, and its biological activities were evaluated. The hybrid compound was found to have somewhat more potent actin-depolymerizing activity than aplyronine A. In contrast, the hybrid compound possessed about 1000-fold less cytotoxicity than aplyronine A. These results indicated that there is no direct correlation between actin-depolymerizing activity and cytotoxicity[11].

The structure of actin in its monomeric form is known at high resolution, while the structure of filamentous F-actin is only understood at considerably lower resolution. Knowing precisely how the monomers of actin fit together would lead to a deeper understanding of the dynamic behavior of the actin filament. Here, a series of crystal structures of actin dimers are reported which were prepared by cross-linking in either the longitudinal or the lateral direction in the filament state. Laterally cross-linked dimers, comprised of monomers belonging to different protofilaments, are found to adopt configurations in crystals that are not related to the native structure of filamentous actin. In contrast, multiple structures of longitudinal dimers consistently reveal the same interface between monomers within a single protofilament. The reappearance of the same longitudinal interface in multiple crystal structures adds weight to arguments that the interface visualized is similar to that in actin filaments. Highly conserved atomic interactions involving residues 199-205 and 287-291 are highlighted[12].

Trisoxazole-containing macrolides and structurally related marine metabolites are widely recognized to exhibit potent and diverse bioactivities such as antifungal activity, cytototoxicity, and icthyotoxicity as well as inhibition of cell division in fertilized sea urchin eggs[4]. The structural uniqueness and potent bioactivity of these compounds have attracted considerable biomedical and synthetic interest. Since the concurrent reports of ulapualides and Kabiramide C from nudibranch egg masses in the late. *Pachastrissa nux* has two distinctive growth forms in one colony, i.e., the protruding gorgonian-shaped capitum and the substratum-attached irregular-shaped base. The sponge has the ability to allocate specifically its major secondary metabolites to the two parts in different levels. Using two cytotoxic trisoxazole macrolides, kabiramides C (2) and G (3), as chemical markers, it was found that the capitum accumulated higher contents of either or both compounds than did the base. However, there were neither inductive nor suppressive correlations among the allocation profiles of either compound in either part of the sponge. The allocation of Kabiramides was a trade-off with the structural materials involved in reinforcing the strength of the sponge.

To date, this is the second report that provides evidence of the specific allocation of bioactive metabolites in two distinctively different organ-like structures in a single sponge colony[13].

A hydrogen bond formed between the carbonyl oxygen of Ala144 and O3 in the ring helps stabilize this interaction. Interactions made by the ring with the opposing actin subunit are colored in green and involve residues Gly23, Asp25, Ile341, Ile345, Ser348, and Leu349. Altogether, these residues compose the binding site of trisoxazole macrolides like Kabiramide C in their 1:1 complex with G-actin[14].

2.2. Ulapualide A and B

Two extraordinary macrolide, ulapaulide3 A (1) and B (Z), which inhibit L1210 leukemia cell proliferation (IC_{50} 0.01-0.03 ~ g/mL) and the growth of *Candida albicadb* apparently are the bioactive metabolites. We report here the gross structures of 1 and 2, 28-membered lactones encompassing three contiguous oxazoles. The acyclic side chains terminate in Nmethylformyl functions but differ in the (2-37 carbonyl of A (l), which in B (2) is an alcohol esterified by 2,3-dimethoxypropanoic acid[15].

2.3. Aplysianin E

Antibacterial and antifungal activities have been found in eggs of the sea hare, *Aplysia kurodai*. The mode of action of an antibacterial glycoprotein was designated and studied by Kisugi *et al*[16],i.e. aplysianin E against a range of bacteria. The growth of both gram-positive and gram-negative bacteria was inhibited and the factors responsible were heat-labile, and sensitive to extreme pH values. Aplysianin E was found to be bacteriostatic but not bactericidal. The synthesis of DNA and RNA by *E. coli* was stopped completely within 4 h of exposure to aplysianin E, suppressing the incorporation of thymidine and uridine.

A single specimen of *Hexabranchus sanguineus*, a nudibranch from the Indo-Pacific that is known to sequester Kabiramides B, C and other trisoxazole macrolides, yielded new kabiramide analogs - 9-desmethylkabiramide B and 33-methyltetrahydrohalichondramide - and two new unexpected thiazole-containing cyclic peptides in sub-micromole amounts. The structures of these cyclic peptides were determined by analyses of 1D and 2D NMR spectra recorded with a state-of-the-art 1-mm 1H NMR high-temperature superconducting micro-cryoprobe, together with mass spectra. In addition to two proline residues, each peptide contains a thiazole- or oxazole-modified amino acid residue, together with conventional amino acid residues. All of the amino acid residues were L- as determined by Marfey's analysis of the acid hydrolysates of the peptides. This is the first report of cyclic thiazole peptides from *H. sanguineus*. Since thiazole-oxazole modified peptides are typically associated with cyanobacteria and tunicates, the finding may imply a dietary component of the *H. sanguineus* that was previously overlooked[17].

2.4. Aplysianin A

L-amino acid oxidase (LAAO) activity, as well as mechanisms of antimicrobial action of Aplysianin A, a sea hare-derived 340 kDa homotetrameric protein, was determined. Spectrophotometric and high-performance liquid chromatography analyses of Aplysianin A indicated that one flavin adenine dinucleotide, a cofactor of LAAO, was bound to each subunit of the homotetramer. Aplysianin A can specifically catalyze oxidation of basic amino acids (L-arginine and L-lysine), and is the first protein from marine invertebrate animals with LAAO activity. Substrate specificity of Aplysianin A is markedly different from that of commonly known LAAO, such as snake venom LAAO, which prefer hydrophobic amino acids. Km value of Aplysianin A was the smallest of those for all known LAAO reported. In the presence of catalase, the antibacterial activity of Aplysianin A was inhibited as expected, indicating that the antibacterial action of aplysianin A results from hydrogen peroxide production during the reaction with substrates. Interestingly, Aplysianin A acted as an antibacterial agent even in the presence of excess catalase. Antibacterial assays in various media suggested that this phenomenon was simply attributed to the consumption of amino acids required for bacterial growth in the media by aplysianin A[18].

2.5. Thisaplysianin E

Thisaplysianin E inhibited the growth of Escherichia coli at a concentration of 0.4 pg protein mL-1 and that of *Sraphylococcus aureus* at 0.13 pg protein. Both antitumor and antibacterial activities were found in all aplysianins[19].

2.6. Tyrian purple

Gastropod mollusks have been used for over 2 500 years to produce the "Tyrine purple " dye made famous by the Phoenicians. This dye is constituted of mixed bromine-substituted indigo and indirubin isomers. Among these, the new natural product 6-bromoindirubin and its synthetic, cell-permeable derivative, 6-bromoindirubin-3′-oxime (BIO), display remarkable selective inhibition of glycogen synthase kinase-3 (GSK-3). Cocrystal structure of GSK-3 β/BIO and CDK5/p25/indirubin-3′-oxime were resolved, providing a detailed view of indirubins' interactions within the ATP binding pocket of these kinases. BIO but not 1-methyl-BIO, its kinase inactive analog, also inhibited the phosphorylation on Tyr276/216, a GSK-3 α/β activation site. BIO but not 1-methyl-BIO reduced β-catenin phosphorylation on a GSK-3-specific site in cellular models. BIO but not 1-methyl-BIO closely mimicked Wnt signaling in Xenopus embryos. 6-bromoindirubins thus

provide a new scaffold for the development of selective and potent pharmacological inhibitors of GSK-3[20]. Only the two cell types with acidophilic granules in the hypobranchial gland showed histochemically strong positive reactions for tryptophan, indicating in these cells high concentrations of the precursors for "Tyrian purple" in gstropod[21]. Although Tyrian purple dye pigments and precursors from muricid molluscs are known for their anti-proliferative and proapoptotic activity, the chemoprotective properties of these edible molluscs have not been assessed. Enhancement of AARGC by oral administration of muricid extract (ME), containing a mixture of the cytotoxins tyrindoleninone and 6-bromoisatin, was assessed in an azoxymethane (AOM) rodent model. A dose-dependent increase in apoptotic index was observed in the distal colon, with a significant increase detected at an ME dose of 1.0 mg/g ($P<0.01$). Proliferation (PCNA) index failed to vary significantly at this ME concentration, which confirms the ME-induced increase in apoptotic response to DNA alkylation. ME also appears to confer no major toxic side effects, as all mice consistently gained weight during the trial and colonic crypt height was maintained ($P>0.05$) independent of ME dose. Although, this is the first example of AARGC enhancement by indole-based compounds, bioactive precursor degradation in simulated gastric fluid may prevent introduction of muricids as a chemopreventative food. Nevertheless, the protective effect of ME against CRC in vivo clearly substantiates further research into the chemopreventative efficacy of Muricidae natural products[22].

The upper quantification limit for 6,6′-dibromoindigotin was improved by over 350%, between standard and optimised systems. Using them, the detection and quantification of trace Tyrine purple components (less than 0.15%) aside from major indigoids becomes possible. Consequently, for the first time, the new analogues of brominated and unbrominated indirubins were found in the shellfish purple from *Hexaplex trunculus*[23].

Anticancer properties of tyrindoleninone and 6-bromoisatin from Dicathais orbita were tested against physiologically normal primary human granulosa cells (HGC) and reproductive cancer cell lines. Tyrindoleninone reduced cancer cell viability with IC50 values of 39 μM (KGN; a tumour-derived granulosa cell line), 39 μM (JAr), and 156 μM (OVCAR-3), compared to 3516 μM in HGC. Apoptosis in HGC occurred after 4 h at 391 μM tyrindoleninone when compared to 20 μM in KGN cells. Differences in apoptosis between HGC and KGN cells were confirmed by TUNEL, with 66% and 31% apoptotic nuclei at 4 h in KGN and HGC, respectively. These marine compounds therefore have potential for development as treatments for female reproductive cancers[24].

Dicathais orbita Tyrian purple inhibits the growth of two marine pathogens, as well as the yeast Candida albicans at 0. 001 mg/mL. Activity against a broad spectrum of marine and human pathogenic bacteria is primarily concentrated in the lipophilic layer of solvent extracts of the spawn. Preliminary GC/MS analyses indicate that the predominant secondary metabolites in these extracts are free fatty acids and sterols[25].

Tyrian purple in the egg masses of the Australian muricid, *Dicathais orbita*. The fresh egg masses contain a high proportion of tyrindoleninone, which reacts to form tyriverdin and subsequently Tyrian purple and 6-bromoisatin as the eggs develop and the larvae hatch. Antimicrobial testing revealed that tyrindoleninone is toxic to both marine and human pathogens at a concentration of 1 mg/mL. Tyriverdin inhibits the growth of two marine pathogens, as well as the yeast *Candida albicans* at 0.001 mg/mL and was effectively bacteriostatic at 0.000 5 mg/mL against three human pathogenic bacteria. Tyriverdin did not appear to significantly lyse the microbial cells. 6-Bromoisatin has mild antimicrobial properties, whereas Tyrian purple exhibited no significant activity. The antimicrobial properties of these compounds and changes in their presence during egg development correlates with ripening in the egg masses of D. orbita. This is the first report of the chemical ripening of eggs in a marine environment[26].

Both gelatinous egg masses and tough egg capsules were found to inhibit microbial growth, suggesting that physical protection alone may not be sufficient to protect the eggs. Antimicrobial activity was observed in the fresh egg masses but not in the well-developed egg masses of a subset of species. The results of this study indicate that a wide range of invertebrates use chemical defense to protect their early stage embryos against bacterial infection[27].

From analysis by gas chromatography/mass spectrometry (GC/MS), the presence of either 2,4,5-tribromo-1H-imidazole (1) or 3,4,5-tribromo-1H-pyrazole (2) was tentatively identified in lipophilic extracts from the egg masses of three muricid molluscs. Synthesis of these compounds, followed by comparison of the GC retention times and fragmentation patterns from electron impact MS, with those of the natural products, indicated that it was 2,4,5-tribromo-1H-imidazole rather than the pyrazole. This imidazole is likely to be responsible for some of the antimicrobial activity observed in the egg[28]. This is the first study to demonstrate that unsaturated fatty acids possess significant bacteriolytic activity against four aquatic pathogens. Encapsulated Anaspidea egg masses contain relatively high concentrations of these unsaturated fatty acids and a lipid mixture modeled on these extracts was strongly bacteriolytic at concentrations down to 0.000 1 mg/mL. Molluscs unsaturated fatty acids and a lipid mixture egg masses contain relatively high concentrations of these unsaturated fatty acids and a lipid mixture modeled on these extracts was strongly bacteriolytic at concentrations down to 0.000 1 mg/mL[25].

6-Bromoindigo (MBI) [systematic name: 6-bromo-2-(3-oxo-2,3-dihydro-1H-indol-2-ylidene)-2,3-dihydro-1H-indol-3-one], $C16H9BrN_2O_2$, crystallizes with one disordered

molecule in the asymmetric unit about a pseudo-inversion center, as shown by the Br-atom disorder of 0.682 (3):0.318 (3). The 18 indigo ring atoms occupy two sites which are displaced by 0.34 Å from each other as a result of this packing disorder. This difference in occupancy factors results in each atom in the reported model used to represent the two disordered sites being 0.08 Å from the higher-occupancy site and 0.26 Å from the lower-occupancy site. Thus, as a result of the disorder, the C-Br bond lengths in the disordered components are 0.08 and 0.26 Å shorter than those found in 6,6′-dibromoindigo (DBI) [Süsse & Krampe (1979). Naturwissenschaften, 66, 110], although the distances within the indigo ring are similar to those found in DBI. The crystals are also twinned by merohedry. Stacking interactions and hydrogen bonds are similar to those found in the structures of indigo and DBI. In MBI, an interaction of the type C-Br C replaces the C-Br Br interactions found in DBI. The interactions in MBI were calculated quantum mechanically using density functional theory and the quantum theory of atoms in molecules[29].

Violet-purple residues collected from a Gallo- Roman burial dated back to the second half of the third century A.D. and excavated at Nintre (France) were chemically investigated by multi-analytical methodology involving the use of Raman spectroscopy, direct exposuremass spectrometry (DE-MS) and high-performance liquid chromatography (HPLC-UV-visible). Little is known about funeral treatment and rituals during Roman times. Retrieving valuable information on these by chemical analysis of organic residues was thus a key aspect of this work. Analyses demonstrated the presence of the very precious purple colorant obtained from shellfish glands commonly known as Tyrian or royal purple and its exceptional preservation. Chemical investigation and archaeological evidence have shown that purple was widely spread after the deposition of the body for burial. These results are the earliest chemical evidence of purple colorant used during funeral rituals (not as textile dye) and enabled us to highlight new aspects of funeral practices in Roman times[30].

Millenniums-old natural dye indigo – a "new" ambipolar organic semiconductor. Indigo shows balanced electron and hole mobilities of 1 Ã – 10 −2 cm 2 V −1 s −1 and good stability against degradation in air. Inverters with gains of 105 in the first and 110 in the third quadrant are demonstrated. Fabricated entirely from natural and biodegradable compounds, these devices show the large potential of such materials for green organic electronics[31].

Murex snails (*murex trunculus*) were popular in antiquity because they were used to create the famed "Tyrian Purple" dye. During the Roman Republic and Empire, the dye was expensive and employed to display social status among the ruling elites. This thesis focuses on the archaeological evidence of Roman murex production, distribution, and consumption, and includes a provisional gazetteer of murex producing sites. Analysis of the data suggests that around the mid 3rd century B.C.E., there was an expansion of murex dye production sites which subsequently contracted during the later Empire. Sites also cluster on the coast of North Africa, and in this region, have the longest period of usage. This thesis argues that the expansion of murex dye sites can be attributed to the influx of agricultural and luxury goods being imported into Rome during the late Republican and early Empire period. During this period, North Africa was also exporting olive oil, fish products, and textiles to Rome. Because the coastal North African cities were already exporting many products to Rome, had the labor and production facilities necessary to create the dye, and also clearly had access to abundant murex snail population, it made sense that North African cities would also add murex dyed textiles to the exports sent to Rome. During the Empire, murex dye continued to grow in popularity, which in fact influenced the contraction of dye sites shown in the gazetteer. This contraction occurred in the 3rd century C.E., during the "Imperial Crisis." The elite were fearful of their status symbol losing its meaning and value because of the increased consumption. As a result, Roman emperors then placed restrictions on the consumption of purple dye, thereby decreasing the number of dye sites needed to supply the Empire[32].

The photophysics of indigo as well as of bispyrroleindigo, the basic chromophore of indigo, has been investigated with ab initio electronic-structure calculations. Vertical electronic excitation energies and excited-state potential-energy profiles have been calculated with the CASSCF, CASPT2 and CC2 methods. The calculations reveal that indigo and bispyrroleindigo undergo intramolecular single-proton transfer between adjacent N-H and C O groups in the 1ϖϖ* excited state. The nearly barrierless proton transfer provides the pathway for a very efficient deactivation of the 1ϖϖ* state via a conical intersection with the ground state. While a low-lying S1-S0 conical intersection exists also after double-proton transfer, the latter reaction path exhibits a much higher barrier. The reaction path for trans H, cis photoisomerization via the twisting of the central C C bond has been investigated for bispyrroleindigo. It has been found that the twisting of the central C C bond is unlikely to play a role in the photochemistry of indigo, because of a large potential-energy barrier and a rather high energy of the S1-S0 conical intersection of the twisted structure. These findings indicate that the exceptional photostability of indigo is the result of rapid internal conversion via intramolecular single-proton transfer, combined with the absence of a low-barrier reaction path for the generation of the cis isomer via trans H, cis photoisomerization[33].

2.7. Unidentified gastropod egg capsules secondary metabolites

The gastropod egg masses are subjected to intense pressure like other marine forms and are also susceptible to predation

and fouling. But, they are reported to have evolved some mechanisms to avoid predation and fouling. By taking into consideration of this unique adaptive strategy, the methanol extracts of the egg masses of the muricid gastropod *Chicoreus ramosus* were screened against ten human pathogenic bacteria. At 10 μg concentration, the extract inhibited the growth of all pathogens except two. Prominent growth inhibition of all the pathogens was observed at a concentration of 75 μg. The highest inhibition was observed against *Escherichia coli* and lowest against *Klebsiella pneumoniae*. The extract showed promising activity against *E. coli*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella paratyphi* and *Serratia marcescens*. The fresh and developing egg masses showed antibacterial activity[34-40].

In view of cross linked nature of the empty capsules could be a potential source of biopolymers that could be suitably used in the field of medicine and also ecofriendly biodegradable polymers[41].

Egg masses of the marine muricid gastropod molluscs *Chicoreus virgineus*, *Chicoreus ramosus* and *Rapana rapiformis* were studied for antifouling activities. The minimum inhibitory concentrations of crude extracts for the inhibition of byssal production and attachment of the brown mussel *Perna indica* were 650 mg/mL, 1 150 mg/mL and 925 mg/mL from the three muricid gastropods, respectively. Higher LC_{50} values than EC_{50} values and 100% recovery of the mussels in the toxicity assay indicated the non-toxic nature of the extracts. The gradient partitioning of the egg mass extracts and subsequent antimicrofouling screening against 40 biofilm bacteria showed wide-spectrum antibacterial activity of the medium polar fraction from *C. virgineus;* the non-polar fraction from *R. rapiformis* and both non-polar and medium polar fractions from *C. ramosus*. The antimicrofouling activity from extracts of the three egg masses was found to be more prominent than antimacrofouling activity. This may be attributed to the targeting of a defense strategy against microbes in order to protect the developing mollusc embryos[42].

The antimicrobial activity of egg masses among 15 molluscan species, among the components of egg masses (gel *vs*. embryos), and among sites where the sea slugs *Haminoea vesicula* and *Melanochlamys diomedea* are located. Egg masses were collected from the field, lyophilized and extracted with ethyl acetate and methanol to isolate non-polar and polar compounds. The extracts were then tested and quantified for antimicrobial activity against marine type cultures (*Bacillus subtillis*, *Vibrio harveyi*, and *Pseudoaltermonas atlantica*), as well as two environmental bacterial isolates, in a Burkholder petri dish and 96-well plate assay. We have observed antimicrobial activity in non-polar and polar extracts of Haminoea and Melanochlamys against Bacillus subtillis and Pseudoaltermonas atlantica, and are continuing with our other comparisons[43].

3. Conclusion

Given all these factors it is not surprising that marine egg capsule has the potential antimicrobial activity, gelatinous egg masses or capsule have evolved several times in invertebrates as a reproductive mode that encapsulates embryos until hatching. The absence of a hard outer covering makes these egg masses particularly susceptible to microbial infection, bio fouling, and predation. The mucus and gel matrix surrounding the egg capsules may contain compounds that deter microbial infection. Furthermore, if adults can adjust the level of protection, then the amount of antimicrobial activity found within an egg mass should be correlated with the bacterial load found in the local environment. To acclimatize the gastropods producing the antimicrobial secondary metabolites with their egg capsule, these compounds are very much minor quantity. The compounds are not enough to characterize as well as clinical trial. So we should culture the organism in corpus or we have been duty-bound to synthesis chemically. In other hand only few compounds have been isolated from marine gastropod egg capsules that also in Atlantic and pacific oceans but in the report on Indian Ocean is limited study has been conducted in marine egg capsule and most of the antimicrobial secondary metabolites has not been characterised from the egg capsule because of the bad odious, and complex chemical structure moreover. Stability of the compounds is unknown.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Burkholder PR, Burkholder LM. Antimicrobial activity in horny corals. *Science* 1958; **127**: 1174-1175.
[2] Hashimoto Y. Marine toxins and other bioactive metabolites. Tokyo: Japan Scientific Societies Press; 1979.
[3] Hadfield MG, Switzer-Dunlap M. Opisthobranchs in the Mollusca. New York: Academic Press; 1984, p. 209-350.
[4] Lord F. Are the contents of egg capsules of the marine gastropod Nucella lapillus (L) axenic? American Malacological Bulletin; 1986, p. 201-203.
[5] Matsunaga S, Fusetani N, Hashimoto K. Kabiramide C, a novel antifungal macrolide from nudibranch eggmasses. *J Am Chem Soc* 1986; **108**: 847-849.
[6] Kamiya H, Muramoto K, Ogata K. Antibacterial activity in the egg mass of a sea hare. *Experientia* 1984; **40**: 947-949.
[7] Matsunaga S. Trisoxazole macrolides from Hexabranchus nudibranchs and other marine invertebrates. *Prog Mol Subcell Biol* 2006; **43**: 241-260.
[8] Petchaprayoon C, Suwanborirux K, Tanaka J, Yan Y, Sakata T, Marriott G. Fluorescent kabiramides: new probes to quantify actin in vitro and in vivo. *Bioconjugate Chem* 2005; **16**: 1382-1389.

[9] Tanaka J. Biomolecular mimicry in the actin cytoskeleton: mechanisms underlying the cytotoxicity of kabiramide C and related macrolides. *Proc Natl Acad Sci USA* 2003; **100**: 13851-13856.

[10] Sirirak T, Kittiwisut S, Janma C, Yuenyongsawad S, Suwanborirux K, Plubrukarn A. Kabiramides J and K, trisoxazole macrolides from the sponge Pachastrissa nux. *J Nat Prod* 2011; **74**: 1288-1292.

[11] Kobayashi K, Fujii Y, Hirayama Y, Kobayashi S, Hayakawa I, Kigoshi H. Design, synthesis, and biological evaluations of aplyronine A-mycalolide B hybrid compound. *Organ Lett* 2012; **14**(5): 1290-1293.

[12] Sawaya MR. Actin dimer cross-linked between residues 191 and 374 and complexed with kabiramide C. *Acta Crystallogr Sect D* 2008: **64**: 454-456.

[13] Sirirak T, Intaraksa N, Kaewsuwan S, Yuenyongsawad S, Suwanborirux K, Plubrukarn A.Intracolonial allocation of trisoxazole macrolides in the sponge Pachastrissa nux. *Chem Biodivers* 2011; **8**(12): 2238-2246.

[14] Klenchin VA, Allingham JS, King R, Tanaka J, Marriott G, Rayment I. Trisoxazole macrolide toxins mimic the binding of actin-capping proteins to actin. *Nat Struct Biol* 2003; **10**: 1058-1063.

[15] Roesener JA, Scheuer PJ. Extraordinary antitumor macrolides from nudibranch eggmasses. *J Am Chem Soc* 1986; **108**: 846-847.

[16] Kisugi J, Ohye H, Kamiya H, Yamazaki M. Biopolymers from marine invertebrates. X. Mode of action of an antibacterial glycoprotein, aplysianin E, from eggs of a sea hare, Aplysia kurodai. *Chem Pharm Bull* 1989; **37**: 3050-3053.

[17] Dalisay DS, Rogers EW, Edison AS, Molinsk TF. Structure elucidation at the nanomole-scale. 1. Trisoxazole macrolides and thiazole-containing cyclic peptides from the nudibranch Hexabranchus sanguineus. *J Nat Prod* 2009; **72**(4): 732-738.

[18] Takamatsu N, Shiba T, Muramoto K, Kamiya H. Molecular cloning of the defense factor in the albumen gland of the sea hare Aplysia kurodai. *FEBS Lett* 1995; **377**: 373-376.

[19] Yamazaki M, Ohye H, Kisugi J, Kamiya H. Bacteriostatic and cytolytic activity of purple fluid from the sea hare. *Dev Comp Immunol* 1990; **14**: 379-383.

[20] Benkendorff K. Bioactive molluscan resources and their conservation: Chemical and biological studies on the egg masses of marine molluscs (Ph.D. thesis). University of Wollongong; 1999.

[21] Naegel LCA, Aguiliar-Cruz CA. The hypobranchial gland from the purple snile Plicopurpura pansa (GOULD, 1853). *J Shellfish Res* 2006; **25**(2): 391-394.

[22] Westley CB, McIver CM, Abbott CA, Le Leu RK, Benkendorff K. Enhanced acute apoptotic response to azoxymethane-induced DNA damage in the rodent colonic epithelium by Tyrian purple precursors: a potential colorectal cancer chemopreventative. *Cancer Biol Ther* 2010; **9**(5): 371-379.

[23] Nowik W, Marcinowska R, Kusyk K, Cardon D, Trojanowicz M. High performance liquid chromatography of slightly soluble brominated indigoids from Tyrian purple. *J Chromatogr A* 2011; **1218**(9): 1244-1252.

[24] Edwards V, Benkendorff K, Young F. Drugs from marine organisms. *Mar Drugs* 2012; **10**(1): 64-83.

[25] Benkendorff K, Davis AR, Rogers CN, Bremner JB. Free fatty acids and sterols in the benthic spawn of aquaticmolluscs, and their associated antimicrobial properties. *J Exp Mar Biol Ecol* 2005; **316**: 29-44.

[26] Benkendorff K, Bremner JB, Davis AR. A putative role for the precursors of Tyrian purple in the egg masses of Australian muricid Dicathais orbita. *J Chem Ecol* 2000; **26**: 1037-1050.

[27] Benkendorff K, Bremner JB, Davis AR. Indole derivatives from the egg masses of muricid molluscs. *Molecules* 2001; **6**: 70-78.

[28] Benkendorff K, Pillai R, Bremner JB. 2,4,5-Tribromo-1H-imidazole in the egg masses of three muricid molluscs. *Nat Prod Res* 2004; **18**: 427- 431.

[29] Szalda DJ, Ramig K, Lavinda O, Koren ZC, Massa L. 6-Bromoindigo dye. *Acta Cryst* 2012; **C68**: 160-163.

[30] Irimia-Vladu M, Głowacki ED, Troshin PA, Schwabegger G, Leonat L, Susarova DK, et al. Indigo-a natural pigment for high performance ambipolar organic field effect transistors and circuits. *Adv Mater* 2012; **24**: 375-380.

[31] Devièse T, Ribechini E, Baraldi P, Farago-Szekeres B, Duday H, Regert M, et al. First chemical evidence of royal purple as a material used for funeral treatment discovered in a Gallo-Roman burial (Naintré, France, third century AD). *Anal Bioanal Chem* **2011**; 401: 1739-1748.

[32] Susmann, Natalie M. The Roman frantic passion for purple" (Pliny 9.66): A geographic analysis of the murex dye industry from the late Roman republican period to late empire. Tufts University; 2011.

[33] Yamazaki S, Sobolewski AL, Domcke W. Molecular mechanisms of the photostability of indigo. *Phys Chem Chem Phys* 2011; **13**: 1618-1628.

[34] Ramasamy MS, Murugan A. Potential antibacterial activity of the chemically defended egg mass of the muricid gastropod Chicoreus ramosus against human pathogens. *Proc Nat Semin Reef Ecosyst Remediat* 2005; **9**: 173-177.

[35] Bragadeeswaran S, Priyadharshini S, Prabhu K, Sophia SR. Antimicrobial and hemolytic activity of fish epidermal mucus *Cynoglossus arel* and *Arius caelatus*. *Asian Pac J Trop Med* 2011; **4**(4): 305-309.

[36] Abirami P, Gomathinayagam M, Panneerselvam R. Preliminary study on the antimicrobial activity of *Enicostemma littorale* using different solvents. *Asian Pac J Trop Med* 2012; **5**(7): 552-555.

[37] Vijayakumar A, Duraipandiyan V, Jeyaraj B, Agastian P, Raj MK, Ignacimuthu S. Phytochemical analysis and *in vitro* antimicrobial activity of *Illicium griffithii* Hook. f. & Thoms extracts. *Asian Pac J Trop Dis* 2012; **2**(3): 190-199.

[38] Moussa A, Noureddine D, Abdelmelek M, Saad A. Antibacterial activity of various honey types of Algeria against Pathogenic Gram-Negative Bacilli: *Escherichia coli* and *Pseudomonas aeruginosa*. *Asian Pac J Trop Dis* 2012; **2**(3): 211-214.

[39] Ravikumar S, Gokulakrishnan R, Boomi P. *In vitro* antibacterial activity of the metal oxide nanoparticles against urinary tract infectious bacterial pathogens. *Asian Pac J Trop Dis* 2012; **2**(2): 85-89.

[40] Jombo GTA, Emanghe UE, Amefule EN, Damen JG. antimicrobial susceptibility profiles at a university hospital in Sub-Saharan Africa. *Asian Pac J Trop Dis* 2012; **2**(1): 7-11.

[41] Kaviarasan T, Siva Sankar R, Yogamoorthi A. Studies in ultra-structure of egg capsule of Chicoreus virgineus and hemifuscus pugilinus using SEM. *J Coast Environ* 2011; **2**(2): 143-151.

[42] Ramasamy MS, Murugan A. Fouling deterrent chemical defence in three muricid gastropod egg masses from the Southeast coast of India. *Biofouling* 2009; **23**(3/4): 259-265.

[43] Smoot SC, Plante C. Antimicrobial activity of molluscan egg masses in the San Juan Islands, Washington. Society for Integrative and Comparative Biology Annual Meeting; 2011, p. 84.