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Bryozoans as a source of new antiparasitic compounds: A systematic review

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ABSTRACT

Due to the lack of effective vaccines and efficient chemotherapy against parasitic infections, the development of antiparasitic innovative medications is an urgent issue. Marine invertebrates were considered as a major source of useful pharmaceuticals. Despite the fact that there has been relatively little research on the bioactive chemicals of the sea-dwelling phylum "Bryozoa", bryozoans have proven to be a rich source of novel therapeutic compounds. In the present article, studies on the antiparasitic effects of chemicals isolated from bryozoans that have been published over the last two decades were reviewed. A total of 26 bryozoans' compounds were investigated as potential antiparasitic compounds from four different genera of Bryozoa. *In vitro* activities of these compounds against five parasites, *Trypanosoma brucei brucei*, *Plasmodium falciparum*, *Leishmania braziliensis*, *Trichinella spiralis*, and *Haemonchus contortus* were reviewed. The challenges of developing these chemicals into functional treatments, as well as the reasons for the lack of research on bryozoans' animals were discussed in the present systematic review.

INTRODUCTION

Globally, the World Health Organization (WHO) predestined that at least 3 billion people worldwide suffer from one or more parasite diseases (WHO, 2013). Unfortunately, vaccines against serious human parasite illnesses such schistosomiasis, malaria, leishmaniasis, and amoebiasis are still lacking (Versteeg *et al.*, 2019). In addition, due to issues with side effects, efficacy, high costs, and the emergence of drug resistance, safe and efficient anti-parasitic medications are still unavailable (Caminade *et al.*, 2018). The major strategy is to investigate innovative medications in the absence of effective vaccinations and chemotherapy.

Marine ecosystem has a higher biodiversity compared to the land that provides numerous resources to human societies (Hill and Fenical, 2010). In order to create novel medicines, invertebrates inhabiting the sea are increasingly being used for screening bioactive marine natural products (MNPs) (Hill and Fenical, 2010). According to Hu *et al.* (2011) marine invertebrates constitute the source of around 75% of the roughly 20,000 MNPs isolated from all marine animals. Invertebrates inhabiting marine environment are

a varied group that may be found in all marine habitats, from the intertidal zone to the deep sea (Thorpe *et al*., 2000).

During exploration natural products for medicinal applications, the invertebrate phylum Bryozoa are considered understudied phylum and few compounds reported from it, in comparable with other invertebrate phyla (Carroll *et al.*, 2020). However, many of these bryozoan compounds having been shown to be bioactive and/or to have unique chemical structures (Tian *et al.*, 2018). According to World Record of Marine Species database (WoRMS, 2021), 6420 extant bryozoan marine species are known. In addition, new taxa are being discovered, particularly in previously unexplored areas like Antarctica and deep sea (Figuerola *et al.*, 2013a). The total number of new bryozoan species described in the literatures from 2005 to 2020 was 932 species, and that described in a single publishing vehicle "Zootaxa" were 330 species in the same period (Gordon & Bock, 2021). Promising biomedically important compounds with a diverse spectrum of biological activities were extracted from bryozoans' animals; Tian *et al.* (2018) reviewed these compounds and their bioactivity significance as follows: macrocyclic lactones with antitumor, enhancing memory and learning, and immune modulatory properties; sterols with cytotoxicity properties; and sphingolipids with moderate cytotoxicity and can be developed as antitumor agents. Besides alkaloids which contain four main groups of β-phenylethylamine alkaloids, indole alkaloids, γ-lactam alkaloids, and pyrrole alkaloids. In addition, other types of alkaloids including indolizine, quinoline, pyridine, isoquinoline, quinolinone, 2,6-naphthyridine, quinone methide, and β-carboline are also recorded. Marine bryozoan alkaloids exhibited variety of biomedical activities including antitrypanosomal, antitumor, anthelminthic, antiviral, antibacterial, antipredator, and antiplasmodial activities, as well as cell division inhibitory effects. In addition to the previous mentioned compounds, the extracted compounds from marine bryozoans are also containing tetracyclic terpenoid lactone and sulphur-containing aromatic compounds, the first exhibited inhibitory activity against metalloprotease collagenase IV and the second exhibited significant antiangiogenic activity and anthelmintic properties.

The objective of the present systematic review is to spotlight on bryozoan extracted compounds with potential antiparasitic properties and discussion of the challenges of developing these chemicals into functional treatments, as well as the reasons for the lack of research on bryozoans' animals.

MATERIALS AND METHODS

The search terms Bryozoa (OR Bryozoan OR Ectoprocta OR Polyzoa), AND Antiparasitic (OR Anthelmintics OR Antihelminthics OR Antiprotozoan) were used in Google Scholar, Scopus and PubMed; to retrieve literature related to the antiparasitic activities of bryozoan crude extracts or their purified compounds. The literature search inclusion criteria were as follows: (1) published between 2000 and October 2022, (2) written in English, (3) available as full text, and (4) classified as original articles. The literature exclusion criteria were as follows: (1) covering synesthetic/semi-synesthetic

substances, (2) compounds isolates from bryozoan-associated organisms, and (3) classified as thesis or letter to the editor. The retrieved articles from databases were preliminary screened, duplicate articles were excluded, the remaining articles were scanned to evaluate the relevance of the content and inclusion-exclusion criteria; and as a result, the most appropriate articles were selected for analysis.

RESULTS

- **Bryozoans' biology and systematic**

In marine, brackish, and freshwater habitats, bryozoa are significant suspension feeders that pull organic food particles out of the water current with their lophophores. They provide as a food source for the nudibranchs and sea spiders that serve as their predators. They provide as habitats for a variety of different small animals, including nematodes, larval mussels, entoprocts crustaceans, and others. Bryozoans typically form encrusting, erect, or massive colonies on solid natural or artificial substrates. Sea weeds, sand grains, and soft deep-sea sediments are all places where it may be found (McKinney and Jackson, 1989). Some bryozoan found as free-swimming discs (O'Dea, 2009).

Bryozoa also known as Ectoprocta, Polyzoa or moss animals or sea mats; they composed of small unites named zooids. Polymorphism (meaning that zooids can be modified morphologically and structurally to perform different functions) is seen in almost all bryozoan organisms. Feeding, reproduction, and defence against micropredators and epizoites are all common zooid tasks. Feeding zooids have a calcified body wall and a soft-bodied component termed the polypide, which is made up of ciliated tentacle crown called lophophore, a gut, body muscles and primitive nervous system. Most bryozoans are considered to be hermaphroditic. In the Bryozoan colonies either zooidal hermaphroditism or zooidal gonochorism (male and female zooids) are present. Both sexual and asexual reproductions are common throughout the life cycle of bryozoan animals. Self-fertilization in bryozoans is assumed to occur only when cross-fertilization is impossible (Ostrovsky, 2020).

Each new colony is started by a sexually generated planktonic larva that lands and metamorphoses into the founder zooid, which then bud additional zooids through asexual reproduction (Gordon, 1977). Bryozoans lack respiratory, circulatory, and excretory systems. Phylactolaemata (freshwater), Gymnolaemata (mainly marine), and Stenolaemata (marine) are the three classes of Bryozoa. There are two orders in the Gymnolaemata: Ctenostomatida (without a calcified skeleton) and Cheilostomatida. Phylactolaemata is the sister group of Gymnolaemata and Stenolaemata, according to modern molecular sequencing methods (Waeschenbach *et al*., 2012).

- **Anthelmintic activity of bryozoan compounds**

The activity of bryozoan compounds as anthelmintic agents was investigated against two parasitic nematode worms *Trichinella spiralis* and *Haemonchus contortus* (Tables 1 & 2). *T. spiralis* affects a wide spectrum of hosts' species including man

causing a disease called Trichinellosis, which is distributed almost all over the world. It affects around 10,000 people each year and has 0.2% mortality rate (Garcı´a *et al.*, 2014). *Trichinella* larvae encysted in muscle tissue of domestic or wild animals are the infective stage of humans and the infection occurred by ingestion. After ingestion of infected meat, the encysted muscle larvae are excysted after being discharged into the host's stomach. The excysted larvae attack the mucosal layer of intestine and undergo 4 moults to reach sexual maturity within 2 to 3 weeks, the fertilized females release about 1500 larvae that penetrate the mucosal layer and carried by the blood and lymph to skeletal muscle where they encysted into infective larvae. The larvae that have been encapsulated in muscle fibres of the host can last for months or even years (Yr and Yf, 2015).

H. contortus is a blood-feeding nematode, produces a sickness that has a significant socioeconomic impact called Haemonchosis/barber's pole. In tropical and subtropical portions of the world, this disease affects ruminants (cattle, sheep, and goats) (O'Connor *et al.*, 2006). Clinically, this disease is distinguished by excessive anaemia and hypoproteinaemia, low quality of carcass, submandibular oedema, decreased wool production, and infected animals may die suddenly. Globally, the economic losses per year in the livestock industry as a resulted of H. contortus infection were estimated by $30-300 million (Roeber *et al.*, 2013 and Emery *et al.*, 2016).

The sexually fertilized female worm produced from 5000 to 15,000 eggs/day. The eggs passed out with faces, within about four to six days, the eggs developed into first larval stage (L1) and second larval stage (L2), and they begin to feed on the bacteria in the excrement. Under favourable conditions, the L2 larvae moult into the infective third larval stage (L3). During grazing, the infective larval stage L3 reaches the abomasum of ruminant animals, usually within 48 hours, it loses its cuticle and burrow into the abomasum's interior layer, where it grows into L4 stage, and finally develops to L5 stage and then to adult worms, which begin feeding on blood (Ehsan *et al.*, 2020).

Eisenbarth *et al.* (2002) isolated three novel disulphides, pentaporins A, B and C, from the Mediterranean bryozoan *Pentapora fascialis* collected from the Mediterranean at depths of 35-45 m. The structures of these compounds were determined by 2D NMR and 1D spectroscopy, EDX-analysis and mass spectrometry. The three pentaporins compounds showed anthelmintic activity against *T. spiralis* in vitro; such activity was referred to the presence of the sulphate ester groups. The authors of this article did not include any information about the concentration at which the compounds harmed the parasites. Narkowicz *et al.* (2002) extracted two new tribrominated alkaloids: convolutamine H and convolutindole A from marine bryozoan *Amathia convoluta*, collected from Tasmania's east coast, Australia. The structures of the two chemicals were investigated by spectroscopic techniques. The nematocidal activity of the two compounds was examined against the free-living larval stages of *H. contortus*. The nematocidal properties shown by extracted novel compounds were more potent than that of available commercial drug "levamisole". While convolutindole A, convolutamine H, and

levamisole have some structural similarities, the authors demonstrate that these compounds do not inhibit nematode growth by the same methods. Levamisole causes characteristic paralysis of nematode larvae, convolutindole A and convolutamine H are lethal to the first and second stage larvae of this parasite. Moreover, Narkowicz *et al*. (2004) screened methanolic extracts of 455 marine macroorganisms (include 28 marine bryazoans: 23 soft& 5 hard) collected from Tasmania's eastern and south eastern coasts, Australia, for their antiparasitic activity against McMaster strain of *H. contortus* (a sensitive reference strain that has had little or no resistance to commercial anthelmintic drugs) and three ectoparasites: fly, mite, and flea. Bryozoan extracts exhibited a significant nematocidal and anti-ectoparasite activity; 35% of the soft (less calcified) bryozoans exhibited high nematocidal activity with LD99<250 µg/ml.

- **Antiprotozoal activity of bryozoan compounds**

The antiprotozoal properties of bryozoan compounds were tested against three parasitic protozoa: *Leishmania braziliensis*, *Trypanosoma brucei brucei* , and *Plasmodium falciparum* (Tables 1 & 2).

Leishmaniasis is one of neglected tropical diseases that are found in 98 countries. Globally, 12–14 million people are estimated to have leishmaniases, and more than 350 million people are at risk of acquiring the illness. Female phlebotomine sand flies of two species, *Lutzomyia* in the New World and *Phlebotomus* in the Old World, transmit *Leishmania* spp (WHO 2010). *Leishmania* spp life cycle include two life stages: within the intestine of the intermediate host, parasites differentiate into metacyclic promastigote stages and migrate to the proboscis of female sand fly. When the infected sand fly bites the mammalian host, it injects promastigotes into its blood vessels; then, the promastigotes are phagocytized by host cells like macrophages, dendritic cells and/or neutrophils; within these cells, the promastigotes differentiate into oval amastigotes. Amastigotes multiply asexually, eventually rupturing the infected cell and attacking other cells. The female sand fly sucks a blood containing amastigotes, completing and repeating the cycle (de Assis *et al.*, 2012). There are two clinical types of leishmaniasis: cutaneous (CL) and visceral (VL). CL is caused by the majority of *Leishmania* species, making it the most prevalent clinical type. It produces skin lesions and ulcers that are typically self-healing. However, mucocutaneous leishmaniasis (MCL), which is more frequently associated with the species *L. braziliensis* and *L. panamensis*, can develop if lesions extend to mucosal areas (WHO 2010).

Bianco *et al.* (2013) tested the antileishmanial activity of hexane, dichloromethane and methanol extracts of marine bryozoan *Bugula neritina* collected from Sambaqui Beach, Brasilia at depth of 1-2 m. The hexane extract and the n-butanol fraction from methanol extract and aqueous residue from methanol extract were active at the concentration of 50 µg/mL against *Leishmania braziliensis* promastigotes, with inhibition values of 66, 47, and 30.7 %, respectively. The hexane extract was poorly effective against intracellular amastigotes with IC50>50 µg/mL.

Trypanosoma brucei brucei infect cattle, but not humans; it differs from *T. brucei rhodesiense* only in the absence of the SRA gene, which helps *T. brucei rhodesiense* to survive in human plasma. However, it is closely related to, and shares fundamental features with the human-infective subspecies (MacLeod *et al.*, 2001 and Sistrom *et al.*, 2016). *T. brucei* are transmitted by tsetse flies. In the fly, *T. brucei* multiply asexually as procyclic forms which differentiate into epimastigotes and then into infectious metacyclic trypomastigotes. The metacyclic stage infects the final host and differentiates into bloodstream "slender" forms that circulate in the blood stream, skin, adipose tissue or the brain. At high density, slender forms differentiate into blood stream "stumpy" (non-multiply) forms, which can be transmitted to the intermediate host when it takes a blood meal (Altamura *et al*., 2020).

Davis *et al.* (2011) purified two new brominated alkaloids, convolutamines I and convolutamines J from the extract of marine bryozoan *Amathia tortuosa* collected at a depth of 63 m by trawling in Bass Strait, Tasmania, Australia. The structures of both compounds were determined after spectroscopic data analysis. Convolutamines I and convolutamines J were evaluated for their antiprotozoal activity against *T. b. brucei*. The first compound appeared to be the more effective against *T. b. brucei*, with an IC_{50} value of 1.1 µM. Convolutamine J exhibits an IC_{50} value of 13.7 µM. The two compounds were investigated for their cytotoxicity using the human embryonic kidney cell line, HEK293; convolutamines I was shown to exhibit cytotoxicity against these cells with an IC_{50} of 22.0 µM, whilst convolutamines J was not active at 41.0 µM. In spite of the antitrypanosomal properties of these compounds, they appeared inactive against Stage II of the infection due to their high molecular weight which prevents their passage through blood-brain barrier.

Six *Plasmodium* species are the etiological agent of human malaria which poses a significant threat to human health: *Plasmodium falciparum*, *P. vivax*, *P. ovale curtisi*, *P. ovale wallikeri*, and *P. malariae*, *P. knowlesi* (Naing *et al*., 2014; Ahmed and Cox-Singh, 2015). Malaria is a serious public health problem, affecting 3.3 billion people in 97 countries and resulting in an estimated 200 million infections and 600,000 deaths each year (WHO, 2015). *Plasmodium* spp. have a complicated life cycle that alternates between female mosquitoes and vertebrate hosts; infection is initiated during the feeding of female anopheline mosquito which injecting sporozoites into the dermis; the sporozoites travel via the bloodstream to the liver, where they undergo through schizogony, releasing tens of thousands of offspring merozoites into the vasculature in packets of merosomes; then they attack the erythrocytes and begin an asexual schizogony cycle in the circulation. A subset of asexually reproducing merozoites is reprogrammed to go through gametocytogenesis. Gametocytes sequester and grow within the bone marrow over the course of 15 days before entering the peripheral circulation and being consumed by mosquitos, in the midgut, they emerge as extracellular male and female gametes. Mating takes place when the micro- and macrogametes fuse to produce a zygote, which

then it changes into an ookinete. Ookinete migrates through the mosquito midgut epithelium and encysts to become an oocyst, where asexual sporogenic reproduction takes place. Oocyst rupture releases motile sporozoites into the haemocoel, which then move into salivary glands, they can then be injected into the next human host (Cowman *et al.*, 2016).

Carroll *et al.* (2011) isolated four alkaloid compounds from marine bryozoans *Amathia wilsoni* collected from Tasmania, Australia; these compounds are two novel alkaloids wilsoniamines A and B and previously known alkaloids amathamides C and H. The structure of these compounds was investigated by MS and NMR analysis. The antiprotozoal activity of the four compounds was tested against chloroquine sensitive (3D7) and resistant (Dd2) strains of *P. falciparum*, and the protozoan parasite *T. brucei brucei.* Generally, amathamides C and H appeared more active than wilsoniamines A and B. The two compounds amathamides C and H exhibited IC_{50} values of 10.2 and 8.0 µM against the Dd2 strain, while they exhibited 28.0 and 14.9 µM IC50 values against 3D7 strain, respectively. Compounds wilsoniamines A and B, on the other hand, only demonstrated growth inhibition against these two strains at substantially greater dosages, with the maximum dose tested (120 µM) achieving 100 percent inhibition. Similarly, only amathamides C and H inhibited *T. brucei brucei* growth to a little extent: IC_{50} values of 32 and 57.1 µM, respectively. The authors did not mention the growth inhibition doses of wilsoniamines A and B compounds against *T. brucei brucei*. In addition, the previous four compounds were tested for their activity to inhibit the growth of human cell cytotoxicity which was assessed using the normal mammalian cell line HEK-293 and the HeLa cancerous cell line; up to and including the greatest concentration measured, none of the substances showed cytotoxicity action (120 µM). The low bioactivity of wilsoniamines A and could be related to a reduction in charged molecule permeability in cells since they are quaternary amine salts. The capacity of amathamide C and H to produce uncharged species may explain their significantly stronger antimalarial activity when compared to wilsoniamines A and B (Carroll *et al.*, 2011).

Carroll *et al.* (2012) isolated a new tribrominated indole alkaloid, kororamide A together with the known alkaloid convolutamine F, through the application of mass directed purification from marine bryozoan, *Amathia tortuosa* collected from northern New South Wales, Australia. The antimalarial activity of kororamide A and convolutamine F were tested against chloroquine-sensitive and resistant strains of *P. falciparum.* Kororamide A was marginally active induced 72% inhibition at 20 µM against the chloroquine-sensitive strain, but less active against the chloroquine resistant strain since it induced 50% inhibition at 20 µM. Convolutamine F was only less active against both strains and only reaching 80% inhibition at the highest dose tested (40 µM). Both compounds were also tested against normal human embryonic cells (HEK) and breast and pancreatic cancerous cells and were shown to be inactive up to a dose of 40 µM.

Caulamidines A and B, trihalogenated alkaloids with an unprecedented heterocyclic structure were extracted from Bryozoan *Caulibugula intermis*. The structures of these compounds were elucidated by use of conventional NMR techniques, computer-assisted structural elucidation (CASE) analysis and DFT-computational studies. Antimalarial activity of the caulamidines was tested against two *P. falciparum* parasite strains, chloroquine-sensitive (D6) and chloroquine-resistant (D7). Caulamidines A and B had significant inhibitory effects on both strains of *P. falciparum*, with IC_{50} values ranging from 8.3 to 12.9 M. Caulamidine A was also examined for cytotoxic activities against NCI-60 cell at a single dose of 40 μM; it displayed very little growth inhibition at this dose, indicating a significant concentration difference between its antimalarial efficacy and cytotoxic effects (Milanowski *et al.*, 2018).

Kleks *et al.* (2020a) purified 11 compounds from the methanol extract of the bryozoan *Orthoscuticella ventricosa* collected from Korora beach, Coffs Harbour, in northern New South Wales, Australia. The structures of these compounds were identified by analysis of MS and 1D and 2D NMR data. The compounds identified as new "bis-β-carbolines and orthoscuticellines A and B (1&2)" that possess a cyclobutane moiety, three new β-carboline alkaloids "orthoscuticellines C−E (3−5)", and six known compounds "1-ethyl-4- methylsulfone-β-carboline (6), 1-ethyl-β-carboline (7), 1-acetyl-β-carboline (8) 1-(1′-hydroxyethyl)-β-carboline (9), 1-methoxycarbonyl-β-carboline (10), and 1-vinyl-β-carboline (11)". Antimalarial activity of compounds 1, 2, and 4-7 was tested against the chloroquine-sensitive 3D7 strain of *P. falciparum*, and for cytotoxicity toward human cells using a human embryonic kidney cell line (HEK293). Compounds 4, 5, 6, and 7 displayed modest antimalarial activity with IC_{50} ranged from 12-21 μM, while compound (1) appeared slightly more potent than other compounds with IC_{50} 10 μM. Compound (2) appeared inactive with IC_{50} more than 40 μM. All tested compounds except compound (1) exhibited non-toxic activity towards HEK293 cell line with IC_{50} > 40 μM.

Kleks *et al.* (2020b) extracted 6 new brominated alkaloids compounds from the bryozoan *Amathia lamourouxi* collected from the rock pools of Woolgoolga, New South Wales, Australia. The isolated compounds are convolutamines K and L (1 and 2), volutamides F−H (3−5), and 2,5-dibromo-1-methyl-1H-indole-3-carbaldehyde (6). The antiplasmodial activity of the 6 compounds was tested against both the chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) parasite strains of *P. falciparum*; and for cytotoxicity toward human cells using a human embryonic kidney cell line (HEK293). The tested compounds 1, 3, 4, and 5 showed moderate to potent antiplasmodial action against both strains of *P. falciparum* strains with an IC_{50} range of 0.57-1.9 μM. Compound 2 exhibited low activity to 3D7 strain with IC_{50} of 11 μM. Compound 6 was inactive against the two parasite strains when tested at 40 μM.

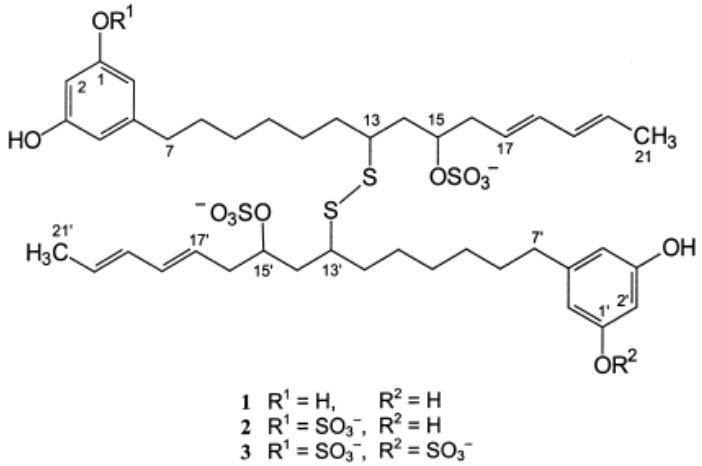
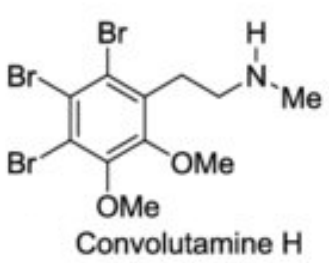
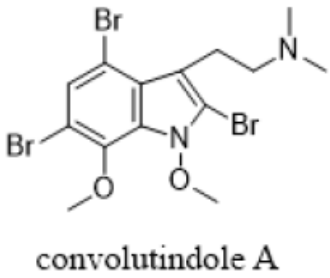
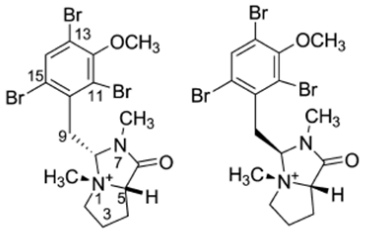
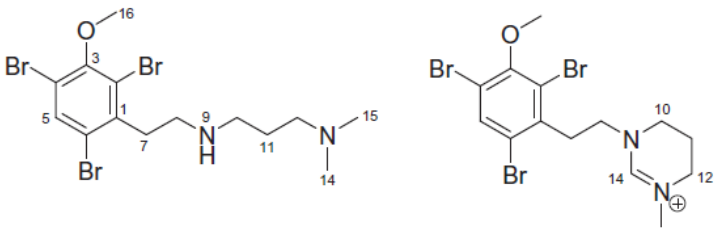
Table (1): Antiparasitic activity of Marine Bryozoans' extracted compounds

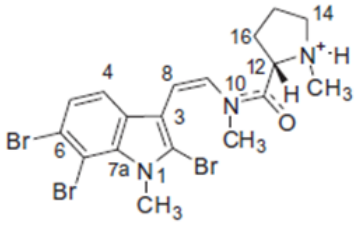
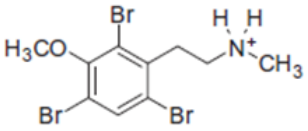
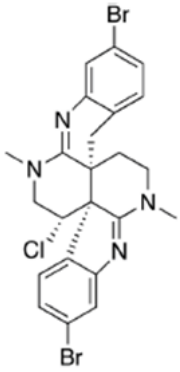
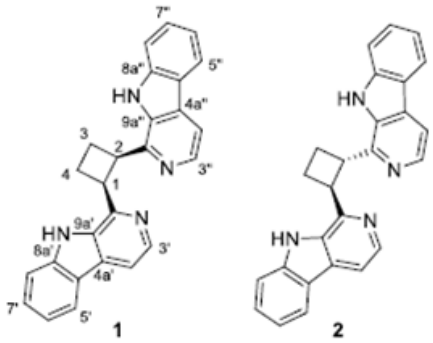
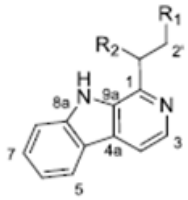
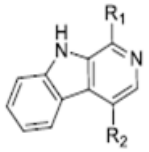
Target parasite	Extracted compounds	Chemistry	IC_{50}	Cytotoxicity Test		Organism	Country/ Region	References
				Type of cells	IC_{50}			
Trichinella spiralis	pentaporin A	disulfidespentaporins	-	-	-	*Pentapora fascialis*	Mediterranean sea	Eisenbarth *et al.* 2002
	pentaporin B							
	pentaporin C							
Haemonchus contortus	convolutamine H	tribrominated alkaloids	LD_{99} 0.2 µg/ml	-	-	*Amathia convoluta*	Australia	Narkowicz *et al.* 2002
	convolutindole A		LD_{99} 0.39 µg/ml					
3D7 *P. falciparum*	wilsoniamines A	alkaloid	120 µM	normal mammalian cell line HEK-293& HeLa cancerous cell line	No cytotoxicity action up to 120 µM	*Amathia wilsoni*	Australia	Carroll *et al.* (2011)
	wilsoniamines B		120 µM					
	amathamides C		28.0 µM					
	amathamides H		14.9 µM					
Dd2 *P. falciparum*	wilsoniamines A		120 µM					
	wilsoniamines B		120 µM					
	amathamides C		10.2 µM					
	amathamides H		8.0 µM					
T. brucei brucei.	wilsoniamines A		-					
	wilsoniamines B		-					
	amathamides C		32 µM					
	amathamides H.		57.1 µM					
T. brucei brucei	convolutamines I	brominated alkaloids	1.1µM	human embryonic kidney cell line, HEK293	22.0 µM	*Amathia tortusa*	Australia	Davis *et. al.* 2011
	convolutamines J		13.7µM		Inactive at 41.0 µM			
chloroquine-sensitive	kororamide A	tribrominated indole alkaloid	72% inhibition at 20 µM	normal human embryonic	inactive up to 40 µM.	*Amathia tortuosa*	Australia	Carroll *et al.* 2012

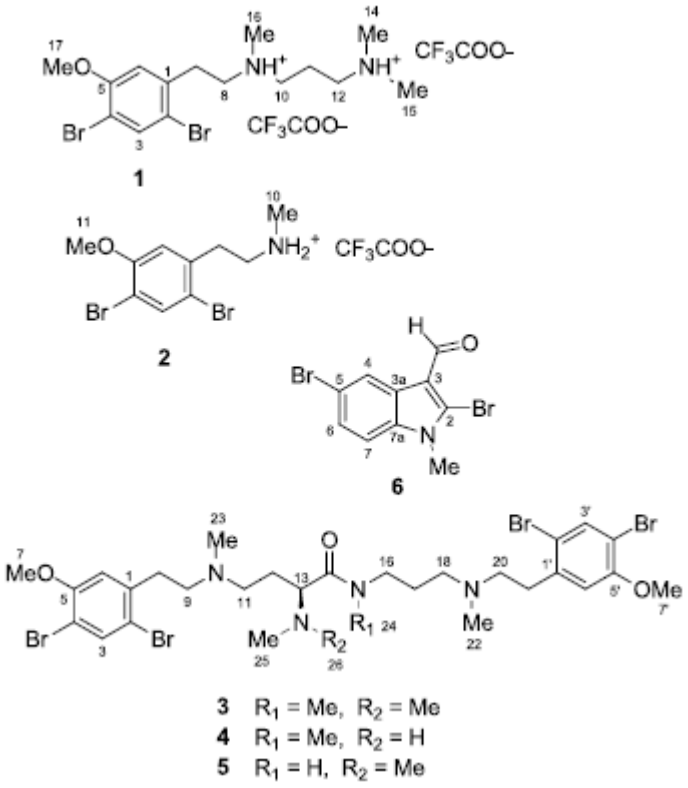
P.falciparum	convolutamine F	alkaloid	50% inhibition at 20 µM	cells (HEK) and breast and pancreatic cancerous cells				
chloroquine-resistant *P.falciparum*	kororamide A	tribrominated indole alkaloid	80% inhibition at 40 µM					
	convolutamine F	alkaloid						
P. falciparum	caulamidines A	trihalogenated alkaloids	8.3-12.9 µM	NCI-60 cell	40 µM	*Caulibugula intermis*	-	Milanowski *et al*., 2018
	caulamidines B			-	-			
3D7 *P. falciparum*	orthoscuticellines A (1)	bis-β-carboline alkaloids	10 µM	human embryonic kidney cell line (HEK293)	10 µM	*Orthoscuticella ventricosa*	Australia	Kleks *et al.*, 2020a
	orthoscuticellines B (2)		>40 µM		>40 µM			
	orthoscuticellines D (4)	β-carboline alkaloids	14 µM		>40 µM			
	orthoscuticellines E (5)		12 µM		>40 µM			
	1-ethyl-4-methylsulfone-β-carboline (6)		21 µM		>40 µM			
	1-ethyl-β-carboline (7)		18 µM		>40 µM			
3D7 *P. falciparum*	convolutamines K (1)	brominated alkaloids	1.7	human embryonic kidney cell line (HEK293)	17.01	*Amathia lamourouxi*	Australia	Kleks *et al.*, 2020b
	convolutamines L (2)		11		>40 µM			
	volutamides F (3)		0.61		>40 µM			
	volutamides G (4)		0.57		11			
	volutamides H (5)		1.6		>40 µM			
	2,5-dibromo-1-methyl-1H-indole-3-carbaldehyde (6)		>40 µM		>40 µM			
Dd2 *P. falciparum*	convolutamines K (1)	brominated	-	human embryonic	17.01			

	convolutamines L (2)	alkaloids	-	kidney cell line (HEK293)	>40 µM			
	volutamides F (3)		0.75		>40 µM			
	volutamides G (4)		0.85		11			
	volutamides H (5)		1.9		>40 µM			
	2,5-dibromo-1-methyl-1H-indole-3-carbaldehyde (6)		>40 µM		>40 µM			

Table (2): The chemical structures of marine Cnidarians' extracted compounds with promising antiparasitic activity of

Compounds	Chemical structures	Authors
pentaporin A (1) pentaporin B (2) pentaporin C (3)	 **1** $R^1 = H$, $R^2 = H$ **2** $R^1 = SO_3^-$, $R^2 = H$ **3** $R^1 = SO_3^-$, $R^2 = SO_3^-$	Eisenbarth *et al.*, 2002
convolutamine H convolutindole A	 Convolutamine H  convolutindole A	Narkowicz *et al.*, 2002
wilsoniamines A wilsoniamines B amathamides C amathamides H	 Wilsoniamine A Wilsoniamine B Amathamide C Amathamide H	Carroll *et al.*, 2011
convolutamines I convolutamines J	 Convolutamine I Convolutamine J	Davis *et al.*, 2011

kororamide A convolutamine F	 Kororamide A  Convolutamine F	Carroll *et al.*, 2012
caulamidines A caulamidines B	 Caulamidine A  Caulamidine B	Milanowski *et al.*, 2018
orthoscuticellines A (1) orthoscuticellines B (2) orthoscuticellines D (4) orthoscuticellines E (5) 1-ethyl-4-methylsulfone-β-carboline (6) 1-ethyl-β-carboline (7)	 **1** **2**  **4** R_1 = OH, R_2 = OH **5** R_1 = SO_3H, R_2 = H  **6** R_1 = Et, R_2 = SO_2CH_3 **7** R_1 = Et, R_2 = H	Kleks *et al.*, 2020a

convolutamines K (1) convolutamines L (2) volutamides F (3) volutamides G (4) volutamides H (5) 2,5-dibromo-1-methyl-1H-indole-3-carbaldehyde (6)	 **1** **2** **6** **3** R_1 = Me, R_2 = Me **4** R_1 = Me, R_2 = H **5** R_1 = H, R_2 = Me	Kleks *et al.*, 2020b

DISCUSSION

Eight papers addressing the antiparasitic characteristics of purified compounds from bryozoans and two publications addressing the antiparasitic activities of bryozoan crude extracts were found in a survey of the literature from 2000 to October 2022. In these articles, only five different genera of bryozoa were examined for their antiparasitic activities, *Bugula*, *Pentapora*, *Amathia*, *Caulibugula*, and *Orthoscuticella*; the most frequently studied genus was *Amathia*. All these genera were collected from Australia except *Bugula* collected from Brasilia and *Pentapora* collected from Mediterranean Sea but the authors did not mentioned the exact site of collection. It is important to mention the source of the bryozoan species to understand the expected variations in the extracted compounds, since Mani *et al.* (2012) reported that the multiplicity of chemicals extracted from the same species can be explained by the diversity of the habitats. During exploration of bryozoan natural product for medical purposes, the most investigated species are those foliose, erect, and large colonies belonging to the order Cheilostomatida (Prinsep *et al*., 2005).

The crude extracts, or the extracted compounds, obtained from bryozoa were tested *in vitro* against five parasites, two parasitic nematodes: *T. spiralis* and *H. contortus* and three parasitic Protozoa: *L. braziliensis*, *T. brucei brucei* and *P. falciparum* (chloroquine-sensitive and chloroquine-resistant strains). The *in vitro* study is usually the

first step in the antiparasitic drug discovery process, since the issue with cellular permeability is confirmed once the extracted material is able to eradicate the parasite (Nweze *et al.*, 2021).

The antiparasitic substances derived from bryozoans belong to two distinct chemical groups: disulphides and alkaloids which performed various biomedical activities (Kuramoto *et al*., 2004& Chen *et al*., 2004). The origin of bioactive compounds present in marine invertebrates like bryozoa are unknown, however they have been demonstrated to derive from food, *de novo* biosynthesis, or symbiotic microorganisms (Lopanik, 2014); the origin of bryozoan compound "bryostatins" has been related to bacterial symbiont *Endobugula sertula* (Davidson *et al.*, 2001 & Lopanik *et al.*, 2004), but it is still unknown for the rest of the compounds (Figuerola& Avila, 2019). Several authors have reported different ecological roles of bryozoan secondary metabolites, including defensive strategies against microorganisms (Figuerola *et al.*, 2017) and abundant and ubiquitous sympatric predators (Figuerola *et al*., 2013b), in addition cytotoxicity against the sea urchin *Sterechinus neumayeri*, because they reduce its reproductive success (Figuerola *et al.*, 2013c).

Prinsep *et al.* (2005) outlined some of the reasons for the paucity of research on bryozoa as follows: (1) insufficient biomass of bryozoan samples allowed for the isolation of secondary metabolites because most species are heavily calcified, (2) the encrusting growth of some bryozoan species lead to technical difficulties for collecting the specimens, and (3) a lack of taxonomic competence and a time-consuming and tedious identification process under the microscope. In addition, there are other challenges related to aquatic environment and invertebrate reported by De Zoysa (2012) as follows: (1) compared to terrestrial sourcing, hydrosourcing is challenging, (2) the large amounts of inorganic salts found in invertebrate extracts is the most reported technical problem, (3) different or even conflicting activities might occur as a result of the presence of several components in a single source, and (4) another major challenge is the extracted bioactive compounds are often found at extremely low concentrations.

Aquiculture of target species might confront obstacles such as limited biomass, difficulties of hydrosourcing, and low concentration of bioactive compounds; also it minimizes the ecophysiological diversity resulting from the variation in environments and habitats. Mendola (2003) reported that estimated economics for commercial-scale marine production of the bryozoan and tunicate were shown to be cost effective; since he succeeded in developing two marine aquaculture systems for the production of huge amounts of biomass for two species of marine animals preferred for their natural chemical contents: *Bugula neritina* (Phylum Bryozoa) and *Ecteinascidia turbinate* (Phylum Tunicata). Furthermore, chemical synthesis and improvement of the techniques used may face the challenges of obtaining pure compounds and avoiding the conflicting activities.

Moreover, taxonomic issues within the phylum bryozoa are believed to be the

cause of the studied species' low diversity (Sharp *et al*., 2007). Bryozoans have been mistaken for plants in the past, and they often found in herbarium collections at museums all over the world. As a result, greater effort should be required to understanding the taxonomy of bryozoans and collecting uncalcified bryozoans and encrusting species, which compete for accessible surfaces and may be predicted to be a rich source of natural compounds (Sharp et al., 2007).

Despite of the scant researches on the bryozoan animals; amazing results were obtained. The two compounds with antimalarial activities: wilsoniamines A and B extracted by Carroll *et al.* (2011) possess a novel ring system that hasn't been found before in nature named hexahydropyrrolo [1,2-c] imidazol-1-one. Moreover, the promising performance of bryozoan natural compounds motivates researcher to synthesis analogues for improvement the compound efficacy. Pham *et al.* (2014) produced synthetic analogues of antitrypanosomal convolutamine I; these analogues are small enough to cross the blood-brain barrier while still being active against *T. b. brucei.*

CONCLUSION

Bryozoa is a very promising invertebrate group in the explorations of new antiparasitic medications; 26 compounds with antiparasitic activity were extracted from only four genera collected from only two countries, thus we can expected the number of compounds that can be extracted from the remaining 6420 extant species, if this group receives enough interest from researchers all over the world. Attempts should be continued to mitigate the challenges limited the hopeful benefits from bryozoa, and scientists should contribute to overcoming the obstacles that have hindered the development of novel antiparasitic drugs from this invertebrate phylum.

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ARABIC SUMMARY

البريوزوا كمصدر للمركبات الجديدة المضادة للطفيليات: مراجعة منهجية

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بسبب نقص اللقاحات الفعالة والعلاج الكيميائي الناجع ضد العدوى الطفيلية، فإن إستكشاف أدوية جديدة مضادة للطفيليات يعد قضية ملحة، وتعتبر اللافقاريات البحرية مصدراً رئيسياً للمستحضرات الصيدلانية المفيدة، على الرغم من أن هناك القليل من الأبحاث حول المواد الكيميائية النشطة بيولوجيًا المستخلصة من حيوانات البريوزوا البحرية، فقد أثبتت البحوث القليلة التي إجريت عليها أنها مصدر غني للمركبات العلاجية الجديدة. في هذا المقال، تمت مراجعة الدراسات حول التأثيرات المضادة للطفيليات للمستخلصات الخام أو المواد الكيميائية المعزولة من البريوزوا التي تم نشرها على مدار العقدين الماضيين. فقد تم إستخلاص ٢٦ مركب مضاد للطفيليات من أربعة أجناس مختلفة من البريوزوا، وقد تمت مراجعة الأنشطة المختبرية لهذه المركبات ضد خمسة طفيليات هي: تريبانوسوما بروسي بروسي، بلازموديوم فيلسباريم ، ليشمانيا برازيلينسيس ، ترايكينيلا سبيرااليس، والهايمونكس كونتورتوس. أيضا في هذه المراجعة المنهجية تمت مناقشة التحديات التي تواجه تطوير هذه المواد الكيميائية إلى علاجات فعالة، وكذلك أسباب ندرة البحوث حول مستخلصات حيوانات البريوزوا ذات الفائدة الطبية والعلاجية.