Molluscicidal activity of some marine substances against the snail Biomphalaria glabrata (Mollusca, Planorbidae)

ARTICLE *in* PARASITOLOGY RESEARCH · DECEMBER 2011

Impact Factor: 2.1 · DOI: 10.1007/s00436-011-2712-x · Source: PubMed

6 AUTHORS, INCLUDING:

Roberto Berlinck
University of São Paulo
131 PUBLICATIONS **2,748** CITATIONS
SEE PROFILE

Eliana Nakano
Instituto Butantan
34 PUBLICATIONS **275** CITATIONS
SEE PROFILE

Lenita Tallarico
University of Campinas and Butantan Instit…
14 PUBLICATIONS **35** CITATIONS
SEE PROFILE

Parasitol Res
DOI 10.1007/s00436-011-2712-x

ORIGINAL PAPER

Molluscicidal activity of some marine substances against the snail *Biomphalaria glabrata* (Mollusca, Planorbidae)

P. A. Miyasato · T. Kawano · J. C. Freitas · R. G. S. Berlinck · E. Nakano · L. F. Tallarico

Received: 8 July 2011 / Accepted: 16 November 2011

Abstract Freshwater snails of the genus *Biomphalaria* play a major role as intermediate hosts of *Schistosoma mansoni*, the etiologic agent of schistosomiasis. While *Biomphalaria* spp. control by molluscicides is one of the main strategies to reduce the snail population in infected areas, there are few effective molluscicides commercially available. Natural products may be considered as potentially useful and safe molluscicides. We have evaluated the molluscicidal activity of 12 extracts from ten marine organisms on adult and embryonic stages of *Biomphalaria glabrata*. Only extracts of the red algae *Liagora farinosa* and of the sponge *Amphimedon viridis* presented molluscicidal activity. Lethal concentration $(LC)_{50}$ values obtained were 120 μg/mL for *L. farinosa* CH_2Cl_2 extract (apolar fraction) and 20 μg/mL for *A. viridis* extract and halitoxin. The polar alga fraction and halitoxin had no effect on *B. glabrata* embryos. The algae apolar fraction was active on *B. glabrata* in all embryonic development stages, with LC_{50} values for blastulae at 42 μg/mL, gastrulae at 124 μg/mL, trochophore at 180 μg/mL, and veliger at 222 μg/mL. This is the first report of extracts from marine organisms which presented molluscicidal activity.

This study is dedicated to the memory of Toshie Kawano.

P. A. Miyasato · T. Kawano · E. Nakano · L. F. Tallarico (✉)
Laboratório de Parasitologia, Instituto Butantan,
Av. Vital Brasil, 1500-CEP: 05503-900,
São Paulo, SP, Brazil
e-mail: letallarico@gmail.com

J. C. Freitas
Departamento de Fisiologia, Instituto de Biociências,
Universidade de São Paulo,
Rua do Matão, Travessa 14, n 321, CEP 05508-900,
São Paulo, SP, Brazil

R. G. S. Berlinck
Instituto de Química de São Carlos, Universidade de São Paulo,
Caixa Postal 780, CEP 13560-970,
São Carlos, SP, Brazil

Introduction

Schistosomiasis is a tropical disease caused by worms belonging to the genus *Schistosoma* (Gryseels et al. 2006). This disease affects more than 200 million people worldwide, with a further 650 million individuals living at risk of infection (WHO 2002). *Schistosoma mansoni* is one of three schistosome species that cause the vast majority of human infections. This parasite is transmitted by *Biomphalaria* snails and found in Africa, the Arabian Peninsula, and South America (Gryseels et al. 2006).

The main targets of the control programs of schistosomiasis are to reduce the morbidity of human host by chemotherapy and the parasite transmission in the snail by the use of molluscicides (WHO 2006). The most effective control of *S. mansoni* has been the use of chemotherapy. However, evidence indicates that resistance to praziquantel, a commonly used *S. mansoni* control agent, may be increasing (Morgan et al. 2001).

The synthetic molluscicide niclosamide is the only effective product and is the molluscicide of choice (WHO 2006). Studies on the molluscicidal activity of natural products have been stimulated due to the high cost of synthetic molluscicides and recurrent resistance of snails to these (Bakry 2009; Bezerra et al. 2002; Luna et al. 2005; Mello-Silva et al. 2007; Rapado et al. 2011; Silva et al. 2006).

Marine organisms have shown to be one of the most promising sources of new bioactive compounds for the treatment of different human diseases (Blunt et al. 2011; Mayer et al. 2011). Although there are no reports on

Published online: 29 December 2011

molluscicidal natural products from marine organisms, such are potentially interesting as sources of secondary metabolites to control the population of intermediate host snails of schistosomiasis.

Some studies with marine products demonstrated the potential as antiparasitic agents. The extract of the alga *Digenea simplex* showed antihelminthic activity against *Ascaris lumbricoides*, *Trichiurus trichiura*, and *Taenia* sp. Subsequently, the neurotoxin kainic acid was isolated and identified as the antihelminthic active compound (Padilla and Martin 1973). Extracts of *D. simplex* have been used as vermifuge medicines (Sakai et al. 2005). In addition to their toxicity to roundworms, kainoids have insecticidal activities against houseflies and cockroaches (Smit 2004). Evaluation of the antileishmanial and antitrypanosomal activity of methanolic crude extracts obtained from cnidarians suggests the potential of natural compounds as antileishmanial drug candidates (Reimão et al. 2008).

The in vitro effect of the aqueous extract from sponge *Amphimedon viridis* on promastigotes of *Leishmania mexicana* suggests that the alkaloid isolated from the sponge can induce cellular lysis of the parasite (Marchán et al. 2000; Tempone et al. 2011). Halitoxins isolated from the sponge *A. viridis* displayed potent hemolytic activity, promoted lysis of sea urchin eggs at very low concentrations, and caused rapid blockade of potential conductance in the nerves of the blue crab, *Callinectes danae* (Berlinck et al. 1996). A complex of halitoxins and amphitoxins isolated from the red sea *A. viridis* displayed selective activity against specific marine bacteria. Halitoxins obtained from the sponge *Callyspongia ridleyi* displayed several changes in membrane transport, such as an irreversible membrane potential depolarization, decreased input resistance, and inhibited evoked action potentials when applied to cultured dorsal root ganglion neuron. The halitoxins of the sponge *Reniera sarai* strongly inhibited acetyl cholinesterase in vitro (Berlinck et al. 2004).

Biomphalaria glabrata represents an important role in the epidemiology of schistosomiasis, with a wide geographical distribution. The normal embryonic development has been described in detail (Camey and Verdonk 1970; Kawano et al. 1992) and showed well-defined and easily recognizable stages allowing the detection of morphogenetic effects. It has been used at the Butantan Institute for more than 30 years to evaluate the mutagenicity and toxicity of chemical (Estevam et al. 2006; Kawano et al. 1979; Kawano and Simões 1986; Nakano et al. 2003; Oliveira-Filho et al. 2010; Rapado et al. 2011; Ré and Kawano 1987) and physical agents (Okazaki et al. 1996; Tallarico et al. 2004). In this work, crude extracts of marine organisms from northern coastline of São Paulo State (Brazil) were assessed to detect molluscicidal and ovicidal properties against *B. glabrata*.

Materials and methods

Marine organisms

Marine organisms (ascidians *Didemnum rodriguesi*, *Didemnum speciosum*, *Microcosmus exasperatus*, *Tridemnum orbiculatum*, and *Phallusia nigra*; algae *Galaxaura marginata* and *Liagora farinosa*; sponges *Axinella* aff. *corrugata*, *A. viridis*, and *Polymastia janeirensis*) were collected by scuba diving, during the summer of 1994 and 1995 in the São Sebastião Channel, northern coastline of São Paulo State, Brazil. Specimens were obtained between depths of 3 and 24 m. After collection, the animals were cleaned with running seawater, immediately frozen, and kept at −20°C until needed.

Crude extracts

Ascidians and sponges species were extracted following a procedure previously reported (Rangel et al. 2001). The algae *G. marginata* and *L. farinosa* and the sponge *A. viridis* were homogenized with MeOH (l:10 *w*/*v*). After filtration, the extracts were concentrated in a vacuum evaporator. The aqueous suspension was partitioned with methylene chloride (CH_2Cl_2). Both the aqueous (polar fraction) and CH_2Cl_2 (apolar fraction) extracts were evaporated to dryness and stored at −20°C.

Isolation and identification of halitoxin

Halitoxin was isolated and identified as previously reported (Berlinck et al. 1996).

Samples preparation

For the apolar extracts, a stock solution containing 1,000 μg/mL of extracts was prepared by suspending the extract in 1% of dimethylsulphoxide (DMSO; Aldrich, Milwaukee, WI, USA) and making up with dechlorinated tap water. For the polar extracts, the stock solutions were diluted with dechlorinated tap water in order to provide assay solutions.

Test organisms

Adults and egg masses were obtained from a population of *B. glabrata* (Say, 1818) originally from Barreiro de Baixo (Minas Gerais, Brazil) and reared under laboratory conditions for several years. Animals were maintained in plastic aquaria (50×23×17 cm) with filtered, dechlorinated, and aerated water and fed with fresh lettuce ad libitum and a balanced diet.

Molluscicidal and egg mass assays

Molluscicidal activities of extracts against *B. glabrata* were determined in agreement to previously described procedures

(WHO 1965). Ten snails, per concentration, with 10–13 mm shell diameter were exposed for each test for 24 h. After exposure, the snails were washed and observed daily for 7 days to record the death rate. The death was ascertained by the absence of heart beatings for 2 min. A pale color of the shell as a consequence of hemolymph loss was also observed after mollusks death. All experiments were repeated three times.

A negative control group was maintained in dechlorinated tap water containing 1% DMSO when necessary, under the same experimental conditions. Niclosamide (Bayluscide WP70®) was used as a positive control. Bayluscide ® WP 70 contains niclosamide ethanolamine salt in the 70% wettable powder formulation (Andrews et al. 1983). Toxicity was assessed by recording the number of viable organisms at different concentrations of tested samples. All results are expressed as lethal concentration $(LC)_{50}$ values, the concentration that affected 50% of the organisms tested in different concentrations of extract.

Extracts were tested first at concentrations of 500 and 100 mg/L. Inactive samples were not further investigated. Definitive assays were conducted with a minimum of four concentrations when a particular extract displayed molluscicidal activity.

Transparent plastic sheets served as substrate for oviposition. Non-damaged egg masses were collected and maintained in Petri dishes with filtered and dechlorinated tap water. Egg masses were observed at a stereomicroscope and classified according to the developmental stage.

Based on the values of LC_{50} for adult molluscs, egg masses with embryos at blastulae (0–15 h after the first egg cleavage), gastrulae (24–39 h), trocophore (48–87 h), and veliger (96–111 h) stages were exposed to specific concentrations of the extracts for 24 h. At the end of exposure, the egg masses were washed with dechlorinated tap water and observed daily for mortality and malformation effects, for 7 days. Embryos without anatomic anomalies, which showed regular development, were classified as normal. Malformed embryos with affected multiple structures were classified as teratomorphic or hydropic, presenting abnormal developmental stage (Geilenkirchen 1966).

A control group was maintained in dechlorinated tap water at the same experimental conditions. Four or five concentrations were employed in the assays and repeated three times with ten egg masses (close to 100 embryos for each concentration). The sum of dead and malformed embryos was used to estimate the LC_{50} values.

Activity assays measured on molluscicidal and egg masses were conducted at 25±2ºC with a 12-h light period. The assays were performed following the World Health Organization procedure (WHO 1965, 1983). Experimental procedures were employed according to accepted principles of animal welfare in experimental science.

Embryos exposed to the CH_2Cl_2 fraction of the alga *L. farinosa* that presented malformation were treated with silver nitrate ($AgNO_3$), as proposed by Holmes protocol (1900). Samples were then rinsed with distillated water, dehydrated with increasing amounts of ethanol, maintained in xilol for 2 min, and embedded in balsam. The same procedure was adopted with embryos of the control group. All embryos were analyzed under Olympus microscope.

Statistical analysis

The median LC_{50} for marine extracts tested in *B. glabrata* adults and embryos and the 95% confidence intervals were estimated using Trimmed Spearman–Karber Method and included in parentheses (Hamilton et al. 1977).

Results

Among the extracts of ten marine organisms studied, only the CH_2Cl_2 extract of *L. farinosa* and the aqueous fraction of *A. viridis* displayed molluscicidal activity against *B. glabrata.* Subsequently, halitoxin isolated from the extract of *A. viridis* was shown to be responsible for the molluscicidal activity observed for the extract of this sponge. The percentage of dead snails in the control with dechlorinated water was 3.33% in all extracts tested. For embryos, the percentage of mortality and malformation observed in the control with dechlorinated water in all developmental stages was below 1.57%. In control maintained with dechlorinated water and DMSO, the percentage of mortality and malformation observed was below 1.51%. The positive control Bayluscide WP70® induced 100% mortality in adults and embryos after 24 h of exposure to 6 μg/mL.

The CH_2Cl_2 extract of *L. farinosa* was active on adults and embryos of *B. glabrata*. On adult snails, LC_{50} was at 120 μg/mL (Table 1). *L. farinosa* extract promoted an increase in the occurrence of lethality and malformed embryos in all stages of *B. glabrata*. The embryos at the veliger stage were five times less sensitive to this extract than embryos at the blastulae stage. The LC_{50} was 42 μg/mL to blastulae, 124 μg/mL to gastrulae, 180 μg/mL to trocophore, and 222 μg/mL to veliger stages (Table 2).

Normal and malformed embryos treated with $AgNO_3$ are shown in Fig. 1. It is possible to verify that the most prevalent abnormality was in apical plate on early stages (blastulae and gastrulae) and in cephalic plate and prototroch in more developed stages (trocophore and veliger).

Snails exposed to the aqueous polar fraction and to halitoxin from the sponge *A. viridis* presented similar responses, with LC_{50} of 20 μg/mL (Table 1). Halitoxin and CH_2Cl_2 extract from *A. viridis* displayed no activity at any stage of embryonic development.

Table 1 Molluscicidal activity on adult *Biomphalaria glabrata* promoted by *L. farinosa* and *A. viridis* extracts and halitoxin

Species	Extracts	Concentration (μg/mL)	Mortality Exp 1	Exp 2	Exp 3	Mean±SD	LC_{50} (μg/mL)[a]
L. farinosa	CH_2Cl_2 fraction	100	3	5	0	2.67±2.52	120 (110–130)
		120	6	7	3	5.33±2.08	
		140	8	4	5	5.67±2.08	
		160	7	9	9	8.33±1.15	
		180	9	10	9	9.33±0.58	
A. viridis	Halitoxin	10	4	2	2	2.67±1.15	20 (14–25)
		20	6	7	6	6.33±0.58	
		30	8	4	6	6±2	
		40	6	7	7	6.67±0.58	
		50	7	8	7	7.33±0.58	
A. viridis	H2O fraction	5	0	0	0	0±0	20 (18–25)
		10	2	1	0	1±1	
		20	7	6	3	5.33±2.08	
		30	7	4	6	5.67±1.53	
		50[b]	10	–	–	–	

Three groups of ten snails were exposed to test extracts for 24 h. All experiments were repeated three times

[a] LC_{50}=50% lethal concentration; n=30 adult snails

[b] n=10 adult snails

Discussion

The marine environment represents a source of organisms that may present useful products awaiting discovery for the treatment of infectious diseases (Donia and Hamann 2003). In this work, screening the molluscicidal potential of organic extracts obtained from the ascidians *D. rodriguesi*, *D. speciosum*, *M. exasperatus*, *P. nigra*, and *T. orbiculatum*, the marine algae *G. marginata* and *L. farinosa*, as well as from the sponges *A.* aff. *corrugata*, *A. viridis*, and *P. janeirensis* revealed that only the apolar extract of *L. farinosa* and the halitoxin and polar fraction of *A. viridis* showed activity against *B. glabrata*.

Extracts obtained from the seaweed *L. farinosa* and *A. viridis* are known for their neuro- and cytotoxicity in several organisms (Berlinck et al. 1996; Freitas et al. 1995, 1996; Mendonça et al. 1997; Williams 1991). Distinct authors observed that aqueous and alcoholic extracts of *A. viridis* presented several potent biological activities, due to the presence of halitoxin in such extracts but also due to the presence of other biologically active compounds. The evaluation of *A. viridis* crude extract bioactivity profile indicated its high toxicity on mouse, sea urchin eggs, and crab (Berlinck et al. 1996). In adult snails of *B. glabrata*, both halitoxin and the polar fraction of *A. viridis* were more active than the apolar fraction of the algae *L. farinosa*. The halitoxin activity proved to be sixfold more potent than the apolar crude extract of *L. farinosa*. Rapado et al. (2011) observed that adult snails released hemolymph when submitted to Piperaceae extracts. The same behavior was observed when the snails were exposed to marine extracts. Such behavior can be associated to the rupture of *B. glabrata* external membranes or by the release of *B. glabrata* hemolymph through the hemal pore (Duncan 1985). Although halitoxin itself is toxic (Berlinck et al. 1996; Berlinck et al. 2004), related 3-alkylpyridinium oligomers proved to be much less toxic than halitoxin (Almeida et al. 1997; Oliveira et al. 2006). Therefore, this structural motif can be useful for the development of molluscicidal agents.

In relation to embryos, the stage of blastula was most sensitive than adults exposed to apolar fraction of *L. farinosa.* The same results were observed in juvenile stages of *Lymnaea stagnalis* that were more sensitive to cadmium exposure than adults (Coeurdassier et al. 2004). Toxicity to *B. glabrata* embryos seems to be associated with particular properties, like solubility, polarity, and size of the molecule, since to reach embryos, chemicals should penetrate the egg gelatinous capsule and cross the egg membrane.

The effect of the apolar fraction of *L. farinosa* on *B. glabrata* blastulae stage was three times more active than in gastrulae and adult snails. When *B. glabrata* was in the trocophore and veliger stages, the concentrations of the apolar fraction of *L. farinosa* four to five times higher than in the blastulae stage were needed to promote the same molluscicidal effect. The veliger stage begins with the development of most adult organs as shell, a beating heart, tentacles, eyes, and foot. All of them can be easily recognized in living larvae (Creton et al. 1993). This increased sensitivity could

Table 2 Effect of *Liagora farinosa* CH_2Cl_2 extract in *Biomphalaria glabrata* embryonic stages

Embryonic stages	Concentration (μg/mL)	Number of embryos	Dead embryos (%)	Malformed embryos (%)	Total (%)	Mean±SD	LC_{50} (μg/mL)[a] (confidence limits)
Blastulae	20	281	14 (5.0)	6 (2.1)	20 (7.1)	6.67±5.03	42 (40–43)
	30	292	32 (11.0)	9 (3.1)	41 (14.1)	13.67±2.08	
	40	288	122 (42.4)	9 (3.1)	131 (45.5)	43.67±37.82	
	50	292	213 (73.0)	7 (2.4)	220 (75.4)	73.33±29.02	
Gastrulae	80	376	50 (13.3)	39 (10.4)	89 (23.7)	29.7±8.32	124 (120–129)
	100	353	63 (17.9)	45 (12.8)	108 (30.6)	32.67±18.03	
	120	390	114 (29.2)	61 (15.6)	175 (44.9)	58.3±21.78	
	140	432	204 (47.2)	73 (16.9)	277 (64.1)	92.3±17.9	
Trochophore	120	351	35 (10.0)	18 (5.1)	53 (15.1)	17.7±18.18	180 (176–182)
	140	312	51 (16.4)	25 (8.0)	76 (24.4)	25.3±3.06	
	160	373	60 (16.1)	31 (8.3)	91 (24.4)	30.3±11.15	
	180	334	123 (36.8)	40 (12.0)	163 (48.8)	54.3±48.81	
	200	365	277 (75.9)	2 (0.6)	279 (76.4)	93±33.60	
Veliger	160	665	37 (5.6)	46 (6.9)	83 (12.5)	27.7±32.3	222 (219–225)
	180	673	146 (21.7)	39 (5.8)	185 (27.5)	61.7±17.5	
	200	725	98 (13.5)	66 (9.1)	164 (22.6)	54.7±9.9	
	220	637	157 (24.6)	152 (23.9)	309 (48.5)	103±87.3	
	240	594	373 (62.8)	26 (4.4)	404 (67.2)	133±23.8	

All experiments were repeated three times

[a] LC_{50}=50% lethal concentration

be attributed to intense cell proliferation activity in the early stages, since it is known that the occurrence of disturbances in these stages can lead to arrested development and inviability of the developing organism (Arner et al. 2009). Previous studies have also shown a differential sensitivity in developmental stages (Kawano et al. 1979; Rapado et al. 2011).

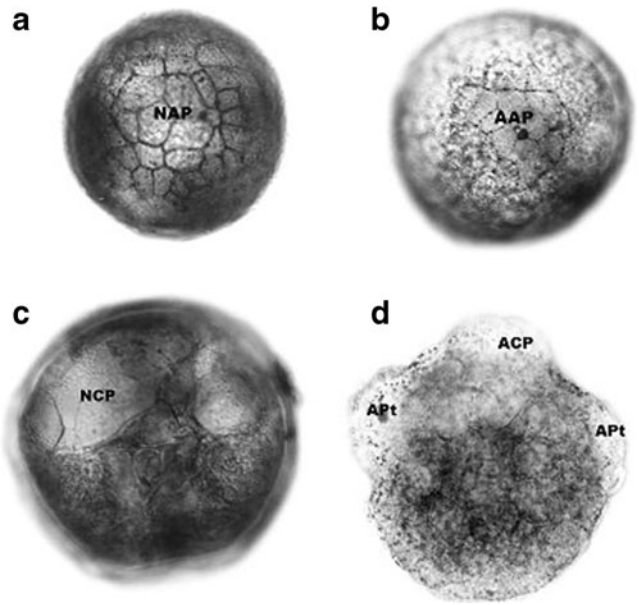

Fig. 1 Embryos exposed to $AgNO_3$. (**a**) Blastulae stage showing normal apical plate with seven cells (*NAP*); (**b**) 24 h after exposition to *Liagora farinosa* CH_2Cl_2 extract, in gastrulae stage exhibiting abnormal development of apical plate with eight cells (*AAP*). (**c**) Trocophore stage showing the normal cephalic plate (*NCP*) and (**d**) trocophore stage after 24-h exposition to *L. farinosa* CH_2Cl_2 extract, cephalic plate (*ACP*) and prototroch (*Apt*) with abnormalities

We observed that snail embryos became abnormal, presenting shell and cephalic malformations with disaggregated cells or hatching delay, when exposed to *L. farinosa* apolar extract, probably due the antimitotic action of this extract previously observed by Mendonça et al. (1997). Oliveira-Filho et al. (2010) observed that a substance that induces embryotoxicity can lead to reduced viability of exposed eggs. Moreover, if these malformed embryos can hatch, a hatching delay itself may damage post-hatching growth and survival and reduce the snail population in an infested region.

In conclusion, the results from the present investigation showed that the apolar extract of the alga *L. farinosa* was toxic to both adults and embryos of *B. glabrata*. Since the apolar extract of *L. farinosa* is lipophilic, it may cross through biological membranes (Mayer et al. 1993). We observed that the LC_{50} value for *L. farinosa* apolar extract was the same when it was applied on adults and embryos at gastrula stage. In finding a molluscicidal agent, it should be active in all snail life stages. Characteristic defects in *B. glabrata* development may occur as a result of treatment during gastrulation. Disturbing processes occurring during this period seem to play an important role in the organogenesis of molluscs (Creton et al. 1993). As for the sponge *A. viridis*, its polar extract and purified halitoxin showed

activity only on *B. glabrata* adults, active at concentrations below 100 mg/L. Despite promoting lysis of cells (Berlinck et al. 1996), both *A. viridis* extract and halitoxin did not show any effect on snail embryos.

This is the first report of molluscicidal agents from marine organisms. Data presented here indicated that the molluscicide action observed requires further investigation in order to unravel possible mechanisms of action for toxicity against *B. glabrata*.

Acknowledgments The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo. The authors thank CEBIMar-USP for facilitating the specimen collection. The authors are grateful to Cristina Antonia Alba de Albuquerque and Alexsander Seixas de Souza for the technical assistance.

References

Almeida AMP, Berlinck RGS, Hajdu E (1997) Alkylpyridine alkaloids from marine sponges. Quím Nova 20:170–185

Andrews P, Thyssen J, Lorke D (1983) The biology and toxicology of molluscicides, Bayluscide ®. Pharmac Ther 19:245–295

Arner J, Vila P, Plautz CZ (2009) Effects of local water contaminants on the development of aquatic organisms. SUJUR 1:12–26

Bakry FA (2009) Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pestic Biochem Physiol 95:159–165

Berlinck RG, Ogawa CA, Almeida AM, Sanchez MA, Malpezzi EL, Costa LV, Hajdu E, Freitas JC (1996) Chemical and pharmacological characterization of halitoxin from *Amphimedon viridis* (Porifera) from southeastern Brazilian coast. Comp Biochem Physiol (C) 115(2):155–163

Berlinck RG, Hajdu E, da Rocha RM, de Oliveira JH, Hernández IL, Seleghim MH, Granato AC, de Almeida EV, Nuñez CV, Muricy G, Peixinho S, Pessoa C, Moraes MO, Cavalcanti BC, Nascimento GG, Thiemann O, Silva M, Souza AO, Silva CL, Minarini PR (2004) Challenges and rewards of research in marine natural products chemistry in Brazil. J Nat Prod 67(3):510–522

Bezerra JCB, Silva IA, Ferreira HD, Ferri PH, Santos SC (2002) Molluscicidal activity against *Biomphalaria glabrata* of Brazilian Cerrado medicinal plants. Fitoterapia 73:428–430

Blunt JW, Copp BR, Munro MHG, Northcotec PT, Prinsep MR (2011) Marine natural products. Nat Prod Rep 28:196–268

Camey T, Verdonk NH (1970) The early development of the snail *Biomphalaria glabrata* (Say, 1818) and the origin of the head organs. Neth J Zool 20:93–121

Coeurdassier M, Vaufleury A, Scheifler R, Morhain E, Badot PM (2004) Effects of cadmium on the survival of three life-stages of the freshwater pulmononate *Lymnaea stagnalis* (Mollusca: Gastropoda). Bull Environ Contam Toxicol 72:1083–1090

Creton R, Zwaan G, Dohmen R (1993) Specific developmental defects in molluscs after treatment with retinoic acid during gastrulation. Develop Growth & Differ 35(3):357–364

Donia M, Hamann MT (2003) Marine natural products and their potential applications as anti-infective agents. Lancet Infect Dis 3:338–348

Duncan J (1985) The toxicology of plant molluscicides. Pharmacol Ther 27:243–264

Estevam EC, Nakano E, Kawano T, Pereira CAB, Amancio FF, Melo AMMA (2006) Dominant lethal effects of 2,4-D in *Biomphalaria glabrata*. Mutation Res 611:83–88

Freitas JC, Mendonça P, Malpezzi ELA (1995) Hemolytic and neurotoxic activities in the extracts of the alga *Liagora farinosa* (Rhodophyta, Nemaliales). Toxicon 33(3):284

Freitas JC, Malpezzi ELA, Costa LV, Berlinck RGS, Almeida AMP, Ogawa CA, Sanchez MAA, Hajdu E (1996) Cytotoxic and neurotoxic effects induced by halitoxin isolated from *Amphimedon viridis* (Porifera). Toxicon 34(3):335

Geilenkirchen WLM (1966) Cell division and morphogenesis of Limnaea eggs after treatment with heat pulses at successive stages in early division cycles. J Embriol Exp Morphol 16(2):321–337

Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368:1106–1118

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11(7):714–719

Holmes SJ (1900) The early development of Planorbis. J Morph 16 (2):369–458

Kawano T, Simões LCG (1986) Efeito da *Stevia rebaudiana* em *Biomphalaria glabrata*. Rev Bras Biol 46(3):555–562

Kawano T, Chaves WR, Simões LCG (1979) Effects of caffeine on embryonic development of *Biomphalaria glabrata*. Cienc Cult 31 (11):1266–1273

Kawano T, Okazaki K, Ré L (1992) Embryonic development of *Biomphalaria glabrata* (Say, 1818) (Mollusca, Gastropoda, Planorbidae): a practical guide to the main stages. Malacologia 34(1–2):25–32

Luna J, de S, dos Santos AF, de Lima MRF, de Omena MC, de Mendonça FAC, Bieber LW, Sant'Ana AEG (2005) A study of the larvicidal and molluscicidal activities of some medicinal plants from northeast Brazil. J Ethnopharmacol 97:199–206

Marchán E, Arrieche D, Henríquez W, Crescente O (2000) In vitro effect of an alkaloid isolated from *Amphimedon viridis* (Porifea) on promastigots of *Leishmania mexicana*. Rev Biol Trop 48 (Suppl 1):31–38

Mayer AM, Paul VJ, Fenical W, Norris JN, Carvalho MS, Jacobs RS (1993) Phospholipase A2 inhibitors from marine algae. Hydrobiologia 260(261):521–529

Mayer AMS, Rodríguez AD, Berlinck RGS, Fusetani N (2011) Marine pharmacology in 2007–8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 153:191–222

Mello-Silva CC, Vilar MM, Bezerra JCB, de Vasconcellos MC, Pinheiro J, Rodrigues ML, de A (2007) Reproductive activity alterations on the *Biomphalaria glabrata* exposed to *Euphorbia splendens* var. *hislopii latex*. Mem Inst Oswaldo Cruz 102(6):671–674

Mendonça P, Malpezzi ELA, Freitas JC (1997) Cytotoxicity of the seaweed *Liagora farinosa* (Rhodophyta, Nemaliales). J Venom Anim Toxins 3(1):199

Morgan JAT, Dejong RJ, Snyder SD, Mkoji GM, Loker ES (2001) *Schistosoma mansoni* and *Biomphalaria*: past history and future trends. Parasitology 123:S211–S228

Nakano E, Watanabe LC, Ohlweiler FP, Pereira CAB, Kawano T (2003) Establishment of the dominant lethal test in the freshwater mollusk *Biomphalaria glabrata* (Say, 1818). Mutation Res 536:145–154

Okazaki K, de Andrade Jr HF, Kawano T (1996) Effect of 60Co gamma radiation on *Biomphalaria glabrata* (Mollusca, Gastropoda) embryos: mortality, malformation and hatching. Braz J Med Biol Res 29(8):1057–1067

Oliveira JHHL, Seleghim MHR, Timm C, Grube A, Köck M, Nascimento GGF, Martins ACT, Silva EGO, de Souza AO, Minarini PRR, Galetti FCS, Silva CL, Hajdu E, Berlinck RGS (2006) Antimicrobial and antimycobacterial activity of cyclostellettamine alkaloids from sponge *Pachychalina sp*. Mar Drugs 4:1–8

Oliveira-Filho EC, Geraldino BR, Coelho DR, De-Carvalho RR, Paumgartten F Jr (2010) Comparative toxicity of *Euphorbia milii*

latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. Chemosphere 81:218–227

Padilla GM, Martin DF (1973) Interactions of prymnesin with erythrocyte membranes. In: Martin DF, Padilla GM (eds) Marine pharmacognosy: action of marine biotoxins at the cellular level. Academic, New York, pp 265–295

Rangel M, de Sanctis B, Freitas JC, Polatto JM, Granato AC, Berlinck RGS, Hajdu E (2001) Cytotoxic and neurotoxic activities in extracts of marine sponges (Porifera) from southeastern Brazilian coast. J Exp Mar Bio Ecol 262:31–40

Rapado LN, Nakano E, Ohlweiler FP, Kato MJ, Yamaguchi LF, Pereira CAB, Kawano T (2011) Molluscicidal and ovicidal activities of plant extracts of the Piperaceae on *Biomphalaria glabrata* (Say, 1818). J Helminthol 85(1):66–72

Ré L, Kawano T (1987) Effects of *Laurus nobilis* (Lauraceae) on *Biomphalaria glabrata* (Say, 1818). Mem Inst Oswaldo Cruz 82 (suppl 4):315–320

Reimão JQ, Migotto AE, Kossuga MH, Berlinck RGS, Tempone AG (2008) Antiprotozoan activity of Brazilian marine cnidarian extracts and of a modified steroid from the octocoral *Carijoa riisei*. Parasitol Res 103:1445–1450

Sakai R, Minato S, Koike K, Jimbo M, Kamiya H (2005) Cellular and subcellular localization of kainic acid in the marine red alga *Digenea simplex*. Cell Tissue Res 322:491–502

Silva TMS, Câmara CA, Agra MF, de Carvalho MG, Frana MT, Brandoline SVPB, Paschoal LS, Braz-Filho R (2006) Molluscicidal activity of *Solanum* species of the Northeast of Brazil on *Biomphalaria glabrata*. Fitoterapia 77:449–452

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Tallarico LF, Okazaki K, Kawano T, Pereira CAB, Nakano E (2004) Dominant lethal effect of 60Co in *Biomphalaria glabrata* (Say, 1818). Mutation Res 561:139–145

Tempone AG, Martins de Oliveira C, Berlinck RG (2011) Current approaches to discover marine antileishmanial natural products. Planta Med 77(6):572–585

WHO (1965) Molluscicide screening and evaluation. In: Bulletin of the World Health Organization, Geneva 33:567–581

WHO (1983) Report of the scientific working group on plant molluscicides. In: World Health Organization, Geneva, 12:1–11

WHO (2002) TDR strategic direction for research: schistosomiasis. In: World Health Organization, Geneva

WHO (2006) Pesticides and their application for the control of vectors and pests of public health importance. In: World Health Organization, Geneva

Williams LAD (1991) Acaricidal activity of five marine algae extracts on female *Boophilus micropus* (Acari: Ixodidae). Fla Entomol 74 (3):404–408