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RESEARCH ARTICLE

Toxicological and Biological Activities of the Acid Secretion of *Berthellina citrina* (Heterobranchia, Pleurobranchidae) from the Red Sea

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Abstract

Berthellina citrina is a pleurobranchid opisthobranch characterized by its skin acid-secretion that contains sulphate and chloride ions with traces of organic matter. To identify defensive potential and bioactivity, we tested this secretion for the first time against *Artemia salina*, different strains of microorganisms, human RBCs and human cancer cell lines. It showed lethality to *A. salina* with LC_{50} values 83.86 and 25.84 µg/ml after 6 and 24 hours, respectively and it caused 100% mortality after 48 and 72 hours. It exhibited antibacterial activity against seven human-pathogenic bacteria, with larger inhibition zone on *Streptococcus pyogenes* and *Staphylococcus aureus* and showed strong inhibition activity against seven fungal species, particularly towards *Paecilomyces variotii*, *Aspergillus flavo-furcatis*, *Fusarium oxysporum* and *Penicillium oxalicum*. It caused significant haemolysis for human RBCs in a range from 38.5 to 77.6 %. Moreover, it showed cytotoxicity against prostate carcinoma cells (PC-3), colorectal carcinoma (HCT 116) and lung carcinoma (A549) with IC_{50} values 6.582, 9.843 and 9.352 µg/ml, respectively. Using HPLC, taurine is the major components of the free amino acids of the secretion with percentage of 52.32%. Thus, the secretion is shown to be highly toxic, bioactive and is interpreted as a chemical defence system against natural predators, but also against fouling. In addition, it might be a new source for bioactive substances that could be used in biomedical research and drugs.

Introduction

Opisthobranchs are less known gastropod molluscs although they show a worldwide distribution from polar regions to the tropical shores and even the deep sea. Only recently they have come into focus in phylogenetics (Kocot et al. 2013). These studies showed that Opisthobranchia are paraphyletic or even polyphyletic (Wägele et al. 2014). Nevertheless, the various "opisthobranch groups" exhibit beautiful colours and extraordinary body shapes, especially those that lack a protective shell. Intriguing is the variety of unique defensive strategies, like sequestration of cnidocysts to protect themselves from predation of fish and crabs (Martin et al. 2007; Obermann et al. 2012). One of the most important strategies is the chemical defence by sequestering secondary metabolites from prey with subsequent accumulation and distribution in their tissues, or *de novo*s synthesis (e.g., Cimino et al. 1999; Putz et al. 2011). Thus they do not only become noxious for their natural predators, but also for the scientist when they release their smelly mucoid toxic substances (Johannes 1963). Recently, several casualties were reported for dogs feeding on the pleurobranchoid *Pleurobranchaea maculata* along beaches in New Zealand. In addition, these slugs had a varying amount of the highly toxic tetrodotoxin in their body (Wood et al. 2012).

Berthellina citrina (Rüppell and Leuckart, 1828) is a very conspicuous opisthobranch belonging to the pleurobranchoid family Pleurobranchidae and thus is a close relative to the genus *Pleurobranchaea*. It is widely

distributed in shallow waters of the Indo-West Pacific region and feeds on a variety of sponges and even corals (Bertsch and Johnson, 1981; Willan, 1984). Many pleurobranchs are characterized by their defensive skin which exudes an acid secretion that contains sulphate and chloride ions with traces of organic matter (Marbach and Tsurnamal, 1973; Thompson, 1983). Indeed, the defensive strategy using sulphuric acid secretion from special epidermis cells are known from further members of other gastropod taxa, including doridoidean Nudibranchia and Cephalaspidea (Heterobranchia) (Thompson, 1988; Gillette et al. 1991), as well as prosobranch families like Cypraeidae and Tonnidae (Caenogastropoda) (Fange and Lidman, 1976; Thompson, 1988). But toxicity has not been shown yet.

Like other marine molluscs, opisthobranchs contain many bioactive substances with antitumor, anticancer, cytotoxic and haemagglutinin activities (Melo et al. 2000; Petzelt et al. 2002; Faircloth and Cuevas, 2006; Shilabin and Hamann, 2011). Also, they contain a wide range of antimicrobial activity, from different parts of their bodies (Lijima et al. 2003; Yang et al. 2005) –just to name a few.

This is the first study that analyses the bioactivity and toxicity of the secretion of a member of the Pleurobranchidae, *B. citrina*. Therefore, the objective of this investigation is to evaluate for the first time *in vitro* the toxicity of the secretion containing sulphuric acid against brine shrimps, antimicrobial activities against different strains of microorganisms, haemolytic activity on human RBCs and the cytotoxic activity against some human cancer cell lines. Moreover, the study aims to estimate the chemical composition of this secretion including amino acids analysis and total protein. The importance of the study on one hand lies in the identification of antifouling and defence mechanisms in the slug against predators, but also in the detection of new marine compounds which might be of interest in medical applications.

Materials and Methods

Animal collection

A total of 50 specimens *of B. citrina* were collected from shallow water along the coast of the Red Sea, 35 km south of Safaga City, Egypt. The samples were transported to the Faculty of Science, Sohag University in convenient containers with sea water for further processing. Identification was verified by Dr. Bill Rudman and by consulting his website (Rudman, 1999).

Extraction of secretion

The animals were washed three times in 3.2% NaCl to remove traces of sea water and then gently dried using absorbent paper. Subsequently the skin was rubbed with a smooth-ended glass rod to stimulate the release of the acid secretion. The animals were then transferred into a clean glass vial and left for 3 minutes (Thompson, 1983). Secretions were collected from the vials and frozen at –20ºC for subsequent tests.

Brine shrimp lethality assay

Dried eggs of brine shrimp, *Artemia salina*, were brought to hatch in flask containing filtered sea water with continuous aeration under continuous light exposure at (25±1°C) and constant salinity (35 PSU). Nauplii (age of 48 h) were transferred to fresh sea water. Twenty individuals were placed in tubes with 1 ml seawater. The toxicity of the secretion was tested by adding different concentration between 10 to 140 µl of the secretion. Three replicates of each concentration were tested and a control was performed with natural filtered sea water. Mortality was scored in each test after 6, 24, 48 and 72 hours. The mean results of brine shrimp mortality were analyzed within a probit analysis (Finney, 1962) and plotted against the logarithms of concentrations using Microsoft Excel. Subsequently, the LC_{50}value with 95% confidence limits (CL) was calculated from the regression equations.

Antimicrobial assay:

Antibacterial activity was determined against seven human pathogenic bacterial strains: four Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus cereus* and *B. subtilis*) and three Gram-negative (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*). Whereas, the antifungal activity was assayed against nine fungal species; the human pathogenic yeast *Candida albicans*, five opportunistic human pathogens (*Fusarium oxysporum*, *F. solani*, *Paecilomyces variotii*, *Aspergillus flavus* and *A. terreus*) and three plant pathogens (*Penicillium oxalicum*, *Cunninghamella echinulata* and *Aspergillus flavo-furcatis*). The clinical fungal species were obtained from Department of Microbiology, Faculty of Medicine, Assuit University, Egypt. Antibacterial and antifungal activities of the secretion were determined by well diffusion method according to the National Committee for Clinical Laboratory Standards NCCLS (1993). Petri dishes containing 20 ml of nutrient broth and Sabouraud's Dextrose Agar medium were inoculated with 50 µl of pathogenic test bacteria or fungi, respectively. Wells (6mm diameter) were made by sterilized cork-borer. 50 µl of the undiluted secretion and 50 µl of the secretion in various concentrations were tested against the tested microorganisms. Various concentrations between 0.25% and 20% were obtained by dilution of the original secretion with dimethylsulfoxide (DMSO) (200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 2.5 µl/ml). DMSO was used as a control. Petri dishes were kept in the refrigerator for two hours until the

extract diffused into the medium and were then incubated aerobically at 37°C for 18-24 h for bacteria and at 28°C for 10-15 days for yeast and fungi. The bacterial and fungal activities were determined by measuring the diameter of inhibition zone and means data are calculated of three trials.

Haemolytic assay

The haemolytic activity of the acid secretion was performed using the method of Haug et al. (2002). Heparinized blood was collected from a healthy person in a tube and RBCs were isolated by centrifugation at 450g for 10 min and washed three times with phosphate buffered saline (PBS) pH 7.4. The test was performed in 96 well U shaped microtitre plates. To each well 40 ml PBS, 10ml of the RBC suspension and various amounts of each test fractions (10, 20 30, 40, 50, 60, 70 and 80 µl respectively) were added. After incubation in a shaker at 37 °C for 1hour the plates were centrifuged at 200g for 5 min. The supernatants were transferred to the microtitre plates and the absorbance at 550 nm was determined spectrophotometrically to measure the extent of RBCs lyses using Plate reader. Positive control (100% haemolysis) and Negative control (0% haemolysis) were also determined by incubating erythrocytes with 1% TritonX-100 (Sigma) in PBS and PBS alone, respectively. Three trials were performed. Statistical analysis was performed using student t-test with a significant level at $p \leq 0.05$. A signed written consent has been obtained from the volunteer individual.

Cytotoxicity assay

Human breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HepG2), lung carcinoma (A549), prostate carcinoma cells (PC-3) and colorectal carcinoma (HCT 116 Cells) were purchased from American type culture collection. Cells were cultured in Dulbeco's modified Eagle's medium (DMEM), whereas HCT-116 cells were grown in McCoy's medium. Media were supplemented with 10% fetal bovine serum (FBS), 2mL glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulfate and 250 µg/ml amphotericin B at 37°C / 5% CO_2. The cytotoxic effect of the *B. citrina* extract on the growth of different human cancer cell lines were determined by SulphoRhodamine-B (SRB) protein method as previously described by Skehan et al. 1990. Briefly, Cells (2 X 10^3) cells/well were incubated for 72 hrs with various concentrations (0, 0.01, 0.1,1,10,100 µg/ml) of the tested extract. After incubation, cells were fixed with 10% trichloroacetic acid and stained with 0.4% SBR for 15 min. The optical density (OD) was measured spectrophotometrically at 540 nm with microplate reader. The half-maximal growth inhibitory concentration (IC_{50}) values were calculated from line equation of the dose response-curve (Sigma Plot software). Fluorouracil (5-FU), a known anticancer drug was used as a positive control.

Chemical composition of *B. citrina* acid secretion

Total protein determination

Total protein concentration of the acid secretion of *B. citrina* was carried out colorimetrically by the method of Bradford (1976), using the bovine serum albumen as a standard.

Amino acid analysis

To quantify the different types of free amino acids (FAA) of *B. citrina* secretion, the collected secretion was lyophilized and sent to the Institut für Technische Chemie, Gottfried Wilhelm Leibniz Universität Hannover, Germany. HPLC (Shimadzu RF-10AxL, Tech lab. LCP4100 pump) was used with flurometric detector Ex 330 Em 420 and water resolve column C18, 5 µm, 3,9x 150 mm using OPA/ MCE reagent (270 mg *o*-phthalaldehyde (OPA) dissolved in 5 ml Ethanol, added to 200 µl mercaptoethanol and 0.4M Borate buffer pH 10 to 50 ml. The sample was prepared first for protein denaturation by adding one part of the sample to four parts methanol and diluted with Borat buffer pH 10.Derivatization by autosampler was carried out using 10 µl sample mixed with 30 µl *o*-phthalaldehyde (OPA) for 1.5 min. (Gnanou et al. 2004).

Results

Brine shrimp assay

The secretion of *B. citrina* showed lethality to *Artemia salina* and the calculated LC_{50}values were 83.86 (CL 6.1-3.3) µg/ml after 6 hours and 25.84 (CL 5.79-2.43) µg/ml after 24 hours. It caused 100% mortality in all applied concentrations after 48 and 72 hours.

Antimicrobial assay

The secretion of *B. citrina* inhibited the growth of all tested human pathogenic bacteria, with considerably larger inhibition zone obtained for *Streptococcus pyogenes* (28 ± 0.404) and *Staphylococcus aureus* (20 ± 0.645) (Fig 1). The secretion of *B. citrina* showed strong inhibition activity against seven fungal species with large inhibition zones on *Paecilomyces variotii* (19 ± 0.361), *Aspergillus flavo-furcatis* (17 ± 0.544), *Fusarium oxysporum* (15 ± 0.450) and *Penicillium oxalicum* (14 ± 0.155). In contrast it had no effect on *Candida albicans* and *Cunninghamella echinulata* (Fig 2).

Haemolytic activity

The secretion of *B. citrina* caused highly significant haemolysis for the human RBCs from 38.5% ($p \leq 0.3X10^{-2}$) to 77.6% ($p \leq 0.19X10^{-3}$) in comparison to 100% haemolysis when applying Triton X100 (Fig 3).

Cytotoxicity assay:

Using SulphoRhodamine-B (SRB) method, the cytotoxic activity of *B. citrina* extract was studied *in vitro* on the growth of five human cancer cell lines. As shown in Table 1, the extract exhibited potential cytotoxic activity (Low IC_{50} value < 10 µg/ml) against prostate carcinoma cells (PC-3), colorectal carcinoma **(**HCT 116) and lung carcinoma (A549) with IC_{50} values; 6 .582, 7.943 and 9.352 µg/ml, respectively. But it had no toxic effect against human breast adenocarcinoma (MCF-7) and hepatocellular carcinoma (HepG2) cancer cells (high IC_{50} value < 10 µg/ml).

Determination of total protein of the acid secretion:

The total protein of the acid secretion of *B. citrina* was determined; equal dl 6.97mg/ml.

Amino acid analysis

HPLC analysis for 20 types of free amino acids (FAA) of *B. citrina* secretion showed that taurine (a sulfur containing amino acid also known as 2-aminoethane sulfonic acid) is the major component of the total amino acid content (52.32%). Arginine also had a relatively high concentration in the secretion (21.95%). Lysine, histidine, cysteine, methionine and tryptophan are absent (Table 2, Fig 4).

Fig 1: Antibacterial activity of secretion of *B. citrina* against some bacterial strains.

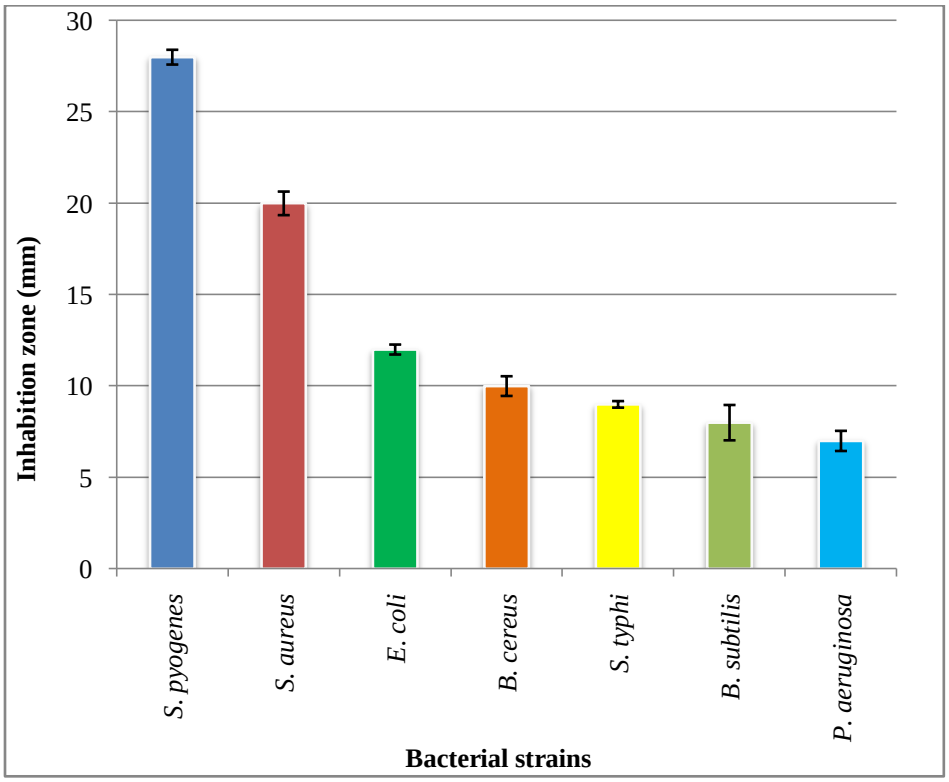

Fig 2: Antifungal activity of secretion of *B. citrina* against some fungal species.

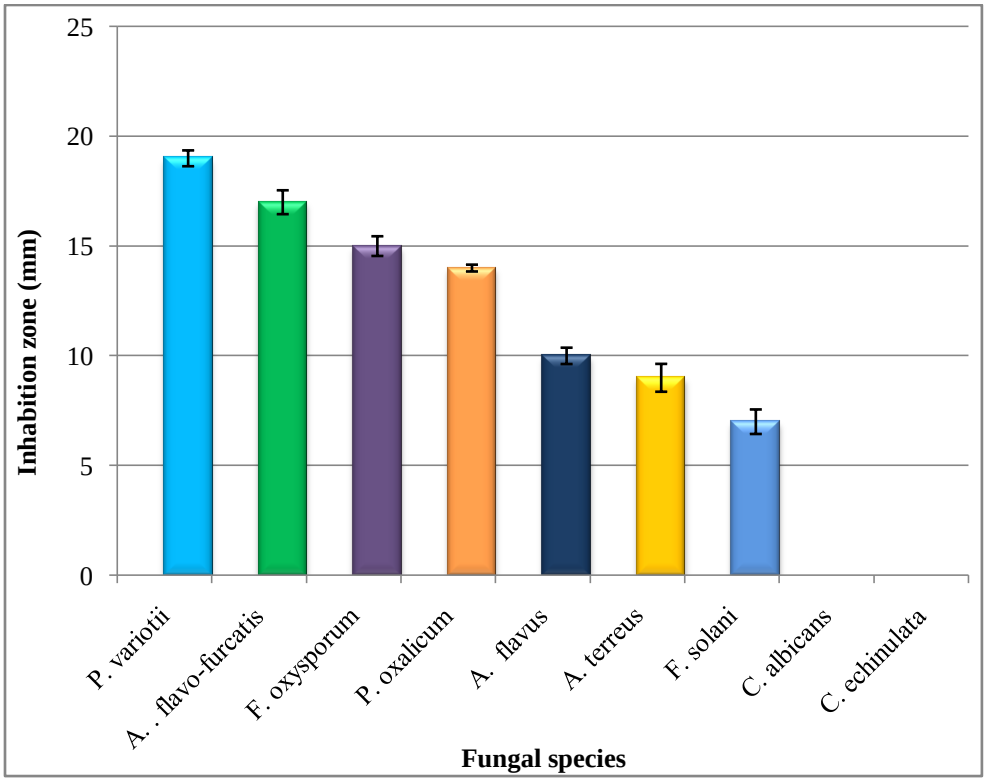

Fig 3: The percentage of haemolysis caused for human RBCs after treatment with different concentrations of the secretion of *B. citrina.*

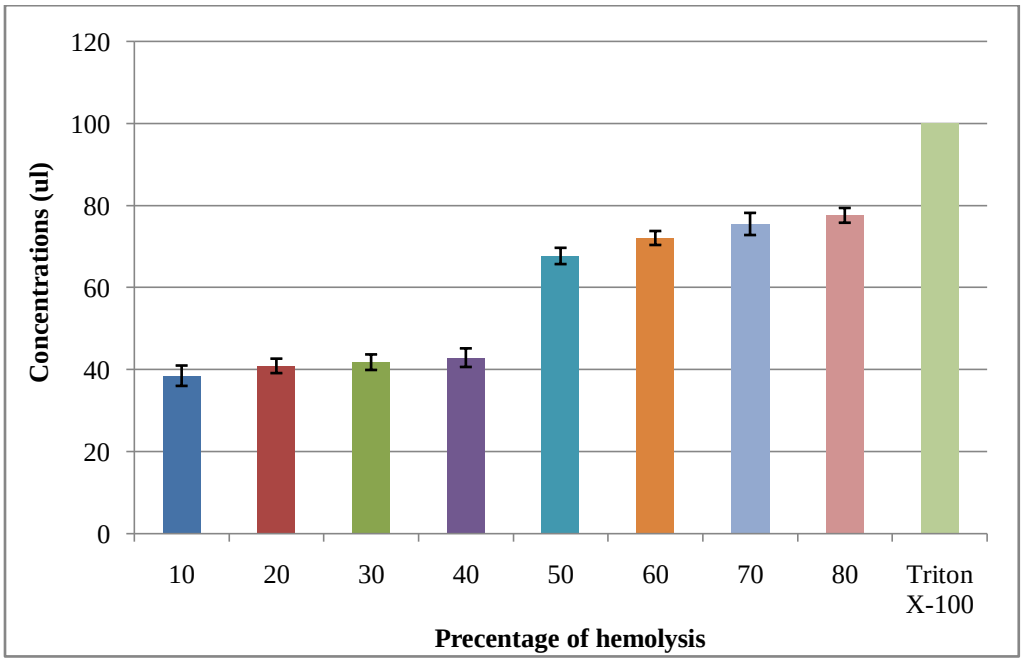

Fig 4: The content and percentage of amino acids in the secretion of *B. citrina*.

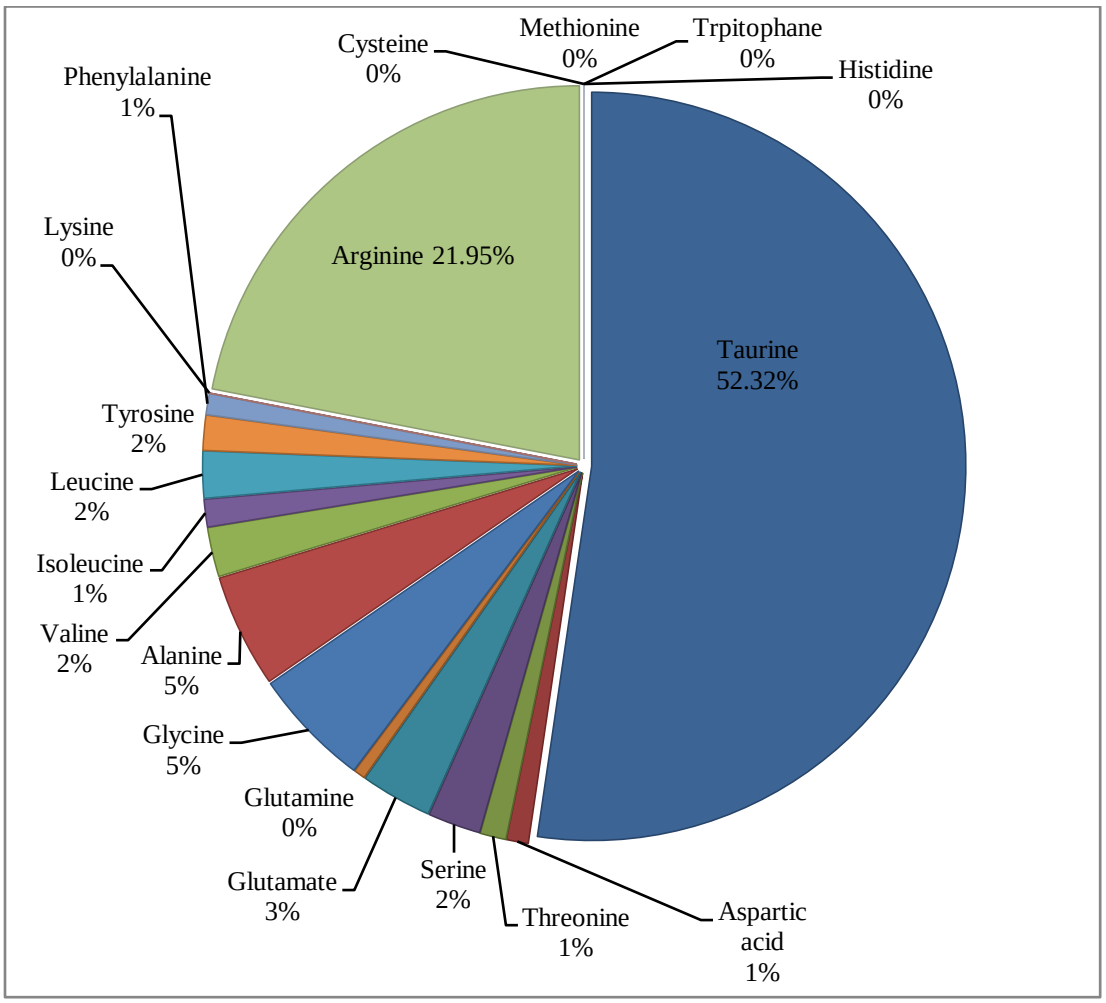

Table 1: Cytotoxic effect of crude extract of *B. citrina* against different human cancer cell lines, using (SRB); data expressed as the mean value of IC_{50} (µg/mL) ± SE. Sample results are compared with 5-FUas a positive control.

	Cell lines				
	PC-3	**A549**	**MCF-7**	**HepG2**	**HCT 116**
***B. citrina* secretion**	6.582±0.947	9.352±1.278	12.087±0.146	13.738±0.625	9.843±1.504
5-FU	0.216±0.0.053	1.127±0.222	0.160±0.007	1.393±0.046	0.594±0.014

Table 2: HPLC analysis of amino acid composition of the secretion of *B. citrina*.

Types of amino acids	molecular weight (mmol/L)
Taurine	3.1392
Aspartic acid	0.05688
Threonine	0.06739
Serine	0.13700
Glutamate	0.18526
Glutamine	0.02947
Proline	-
Glycine	0.30846
Alanine	0.29387
Valine	0.12794
Isoleucine	0.07142
Leucine	0.12138
Tyrosine	0.09045
Phenylalanine	0.05412
Lysine	0
Histidine	0
Arginine	1.31731
Cysteine	0
Methionine	0
Tryptophan	0
Total amino acids	6.00015
- (not detected)	(0) absent

Discussion

Marine organisms represent a wide source for bioactive natural compounds that can be evaluated with regard to drugs activity. Many compounds have already been isolated from marine invertebrates, especially cnidarians, sponges, ascidians, bryozoans and molluscs. Some of these compounds are now being used in clinical treatment (Newman and Cragg, 2004; Faircloth and Cuevas, 2006). In the present study, we report for the first time the toxicity and biological activity of an acidic secretion, with which a member of the taxon Pleurobranchidae defends itself. According to our results, the species *B. citrina* exhibited a high toxicity against a crustacean, some human cancer cell lines and a wide spectrum of antibacterial and antifungal activity.

In the present study the secretion of the slug exhibited high toxicity to *A. salina* with LC_{50}= 83.86 µg/mL and 25.84 µg/mL after 6 and 24 hours, respectively. Notably, the secretion caused 100% mortality for all concentrations after 48 and 72 hours. Several extracts of opisthobranchs were toxic to *A. salina* including the ink fluid of the anaspidean *Aplysia dactylomela* with LC_{50} value 141.25 µg protein/ml (Melo et al. 1998). Secondary metabolites of the cephalaspidean *Philinopsis depicta* with LC_{50} value 33.7 µg/ml (Marin et al. 1999). The whole body extracts of the doridoidean nudibranch *Roboastra gracilis* and the cephalaspideans *Chelidonura inornata* and *Sagaminopteron ornatum* were found to cause 100% lethality, whereas the extract of the sacoglossan *Elysia ornata* caused 27% mortality (Cortesi and Cheney, 2010). The whole body extract of the sea hare *Bursatella leachii* with LC_{50} value 25 µg/ml (Kanchana et al. 2014).

High antibacterial activity was found against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli*. Most bacterial strains grow only in a pH range of 5-9 (Madigan et al. 2009) and very low pH values have detrimental effects on bacterial strains (Mason et al. 2006). This may explain the wide spectral activity against both the Gram-negative and Gram-positive human pathogenic bacteria of the secretion of *B. citrina* in the present work, since most of the secretion is composed of sulphuric acid with a pH value of around 1 (Marbach and Tsurnamal, 1973, Thompson, 1988). Marine molluscs contain a wide range of antibacterial substances, among opisthobranchs, anti-bacterial activities were mainly reported for sea hares (Anaspidea). Yamazaki et al. (1990) mentioned that the glycoprotein contents of the ink fluid of the sea hare *Aplysia kurodai* inhibited *S. aureus*, *E. coli* and *Salmonella enterica*. Also, Melo et al. (2000) purified a protein from the ink of *A. dactylomea* with antibacterial

activity against S. *aureus*, *P. vulgaris* and *P. aeruginosa*. A novel peptide isolated from the ink of *Dollabella auricularia* inhibited the growth of *E. coli*, *S. aureus*, *Haemophilus influenza*, *B. subtilis*, *Vibrio vulnificus* and *Listeria monocytogenes* (Lijima et al. 2003). A monomeric protein isolated from the ink of *Aplysia californica* inhibited the growth of *P. aeruginosa*, *S. aureus*, S. *pyogenes* and *Vibrio harveyii*, (Yang, et al. 2005). Moustafa (2005) reported that the ink of *Aplysia oculifera* and *A. fasciata* inhibited the growth of *S. aureus* and *E. coli*. In addition, an isolated dimericisoquinoline alkaloid from the nudibranch *Jorunna funebris* showed antibacterial activity against *B. subtilis* and *S. aureus* (Cimino et al. 2001).The egg masses of many heterobranchs showed antibacterial activity against some marine and human pathogenic bacteria (Benkendorff et al., 2011). The whole body extract of sea hares *Aplysia* sp. *Bursatella leachii* and nudibranch *Kalinga ornate* caused inhibitory effects against seven fish pathogens (Sadhasivam et al., 2013).The whole body extract of the sea slug *Armina babai* showed a wide range of activity against the pathogens incrude extract as well as fractions (Ramya et al. 2014).

In the present investigation, a broad spectrum of antifungal activity of *B. citrina* with higher values of inhibition zones against *Paecilomyces variotii*, *Aspergillus flavo-furcatis*, *Fusarium oxysporum* and *Penicillium oxalicum*. The broad spectrum of antifungal activity may again be due to the presence of sulphuric acid in the secretion. Although fungi can grow at a pH value of 3.3-6.3 (Hansen, 2008), the lower pH value in the secretion of pleurobranch species (around 0.8 to 1.0), according to Marbach and Tsurnamal (1973) and Thompson (1988) might be beyond the tolerable limit for the fungi tested here. A great spectrum of antifungal activity has been reported for several members of opisthobranchs. The nudibranch *Hexabranchus sanguineus* contains antifungal substances derived from its prey sponge (Kernan et al. 1988). The egg mass of the anaspidean sea hare *Aplysia kurodai* showed antifungal activity against *C. albicans* (Kisugi et al. 1989). Iijima et al. (1995) isolated Aplysianin-E from *Aplysia sp.*, which inhibited the growth of the yeast species *Saccharomyces cerevisiae*, *Schizosaccharomyces sp.* and *C. albicans.* Moustafa (2005) reported that ink of *Aplysia oculifera* and. *A. fasciata* inhibited the growth of *F. oxysporum*. The methanolic extract of the whole body of *Bursatella leachii* inhibited the growth of *Fusarim* sp. and *Aspergillus fumigatus* (Kanchana et al. 2014). The sacoglossan *Elysia rufescens* accumulates cyclic dipeptides from the green alga *Bryopsis pennata* which has antifungal activity against *C. albicans*, *C. neoformans* and *A. fumigatus* (Shilabin et al. 2007).

In the present work, the secretion of *B. citrina* caused haemolysis to human RBCs in a range from 38.5 to 77.6%. The haemolytic activity of the secretion may be due to the presence of sulphate and chloride and the low pH value of the secretion. According to Bodansky (1928), inorganic acids cause haemolysis by inducing decomposition of the corpuscle that is rather due to the injury of the cell membrane than due to an osmotic effect. Among Heterobranchia, only the anaspidean sea hares have been recorded to contain substances with haemolytic activity, like the ink of *Aplysia oculifera* and *A. fasciata* caused haemolysis on human RBCs with a range from 31% to 96% and from 29% to 32%, respectively (Moustafa, 2005). The ink of *Dollabella auricularia* showed 100% lyses of chicken RBC (Vennila et al. 2011).

The present data revealed that the extract of *B. citrina* secretion exhibited cytotoxic activity (Low IC_{50} value < 10µg/ml) against prostate carcinoma cells (PC-3), colorectal carcinoma **(**HCT 116) and lung carcinoma (A549) with IC_{50} values; 6.582, 9.843 and 9.352 µg/ml, respectively. Heterobranchs are a rich source of many of cytotoxic, anticancer and antitumor substances. Among these substances; two anticancer agents in human clinical trials known as dolastatins and kahalalide F isolated from the sea hare *Dolabella auricularia* and the sacoglossan *Elysia rufescens*, respectively. They were reported to have cytotoxic effects against breast cancer, lung cancer, leukemia, and lymphoma (Shilabin and Hamann, 2011; Serova et al. 2013). Within nudibranchs, cytotoxic sesterterpenoids and scalaranes were isolated from *Chromodoris inornata* (Miyamoto et al. 1999). A cytotoxic Trisoxazole macrolide isolated from the egg-ribbons of *Hexabranchus* (Matsunaga, 2006). Extracts from *Asteronotus cespitosus*, *Halgerda gunnessi* and *H. aurantiomaculata* showed cytotoxicity against P388 murine leukemia cell lines (Fahey and Carroll, 2007). Cytotoxic isonitrile lipid on tumor mammalian cells was reported from *Actinocyclus papillatus* (Manzo et al. 2011). Within anaspidean sea hares, a protein known as cyplasin isolated from the mucus of *Aplysia punctat* showed cytotoxicity against rat cell line PtK2 and human melanoma cell lines (Petzelt et al. 2002). D-galactose binding lectin of the eggs of *Aplysia kurodai*s showed cytotoxicity against Burkitt's lymphoma Raji cells and erythroleukemia (Kawsar et al. 2011). Isolated fractions of fatty acid composition of the cephalaspidean *Scaphander lignarius* have cytotoxicity against melanoma, colon carcinoma and breast carcinoma (Vasskog et al. 2012**)**. Moreover, new halogenated sesquiterpenes from the sea hare, *Aplysia oculifera* exhibited cytotoxic activity against several human cancer cell lines (Hegazy et al. 2014).

Thompson and Slinn (1959) reported that the acid secretion of *Pleurobranchus membranaceus* had only sulphate and chloride ions with no proteins. Our findings contradict these results. However, the acid secretion of *B. citrina* contains rather low amounts of proteins (0.697 mg/ml).This finding would explain the presence of batches of

proteinous substances as light-bluish stains in the content of large acid glands within the histological sections of the skin of *B. citrina*, which were described by Wägele et al. (2006).

Interestingly, taurine constituted the major component of the FAA of *B. citrina* secretion. This result is in accordance with other molluscan defensive secretions as in sea hares and cephalopods (Kicklighter et al. 2005; Derby et al. 2007). This certainly confirms the idea that the major predators of these molluscs (e.g. crustaceans and fishes) are highly sensitive for these FAA and have specialized receptors to detect them (Caprio, 1988). It strongly supported the potential function of these defensive secretions as sensory disruptors of molluscan predators or a type of phagomimicry (Kicklighter et al. 2005, Derby et al. 2007). Moreover, the metabolism of sulphur containing compounds like taurine may be one of the factors that is responsible for the formation of sulphate ions in mollusks (Fange and Lidman, 1976). Also, taurine has a role in sulfide detoxification as it serves as sulfur storage compound (Joyner et al., 2003). Noteworthy, molluscan tissues have high concentrations of taurine as it plays a biological role in tissue osmoregulation and larval development (Welborn and Manahan, 1995).

In conclusion, the present study provided the first evidence that the secretion of *Berthellina citrina* is highly bioactive, which certainly serves the animals against putative predators. The high amount of the feeding stimulant taurine in the secretion attracts the predator and thus distracts it from the slug. This secretion might be a new source of bioactive substances that can be used in biomedical research as it exhibited cytotoxic activity against prostate, lung and colorectal carcinoma and antimicrobial activity on human pathogenic bacteria and fungi. Moreover, it showed haemolytic activity on human RBCs and had toxic effects to lower metazoan organisms.

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