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Antimicrobial Activity of Protein Hydrolysate from Marine Molluscs *Babylonia spirata* (Linnaeus, 1758)

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ABSTRACT

The present study is to investigate the antimicrobial activity of protein hydrolysate of marine water mollusks *Babylonia spirata* (Linnaeus, 1758). Protein hydrolysate was prepared from tissue of *Babylonia spirata* by enzymatic hydrolysis. Enzyme digestion were carried out with the enzyme Trypsin. The protein concentration was estimated by Bradford's method and the protein quantification was done by using SDS PAGE analysis. Antibacterial assay was carried out against four bacterial pathogens by agar well diffusion method and antifungal activity was performed against three human pathogenic fungal strains. 2.6mg/ml protein concentration was estimated by Bradford's method and 40 to 200 kDa protein bands were resulted in SDS PAGE analysis. In antimicrobial activity, the maximum zone of inhibition was observed against *Staphylococcus aureus*22.16 $\pm$1.04mm at 1000µg/ml concentration and the maximum zone of inhibition was observed in *Aspergillus fumigatus*13.5$\pm$0.5 in 1000µg/ml concentration. These results are signify that the protein hydrolysate of marine molluscs *Babylonia spirata* express remarkable antimicrobial activity.

INTRODUCTION

The marine environment is a huge source to discover bioactive natural products. The number of natural products are isolated from marine organisms increases rapidly, and now exceeds with hundreds of new compounds being discovered every year (Faulkner, 2002; Proksch and Muller, 2006). Bioactive compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and mollusks (Proksch *et al*., 2002). Marine invertebrates offer a source of potential antimicrobial drugs (Bazes *et al*., 2009). The majority of research on natural products from the phylum Mollusca has been focused on primarily soft-bodies or shell-lessmolluscs, particularly nudibranches and opisthobranches (Karuso, 1987; Faulkner, 1992). However some studies have also been reported biological activity from shelled molluscs (Kumar, 2011; Kumaran *et al*., 2011). Many bioactive compounds have been investigated predominantly for their antimicrobial, cytotoxic, anti-tumor and anti-inflammatory, anti- leukemic, antineoplastic and antiviral properties of molluscs (Anand and Edward, 2002; Kamiya *et al*., 1989; Pettit *et al*., 1987; Kisugi *et al*., 1989; Rajaganapathi *et al*., 2000). Generally fewer extensive, investigations have been made of the antimicrobial proteins of molluscs groups and although whole body homogenates of some marine molluscs have been reported for antimicrobial compounds. Studies of antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries. Antimicrobial peptides are important in the first line of the host defense system of many animal species (Boman, 1995). Their value in innate immunity lies in their ability to function without either high specificity or memory. Moreover, they are synthesized without dedicated cells or tissues and they can rapidly diffuse to the point of infection.

The potential of marine gastropod as a source of biologically active products is largely explored in India. Therefore, the aim of the present study was to evaluate the antimicrobial activity of the tissue extracts of gastropod *Babylonia spirata* against different pathogenic bacterial and fungal strains.

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MATERIALS AND METHODS

Collection and identification

Babylonia spirata (Linnaeus, 1758) Marine water snails were collected from, Kasimedu landing centre, Chennai, Tamil Nadu, India. They were identified by Dr. R. Venkitesan, Scientist - C, Zoological Survey of India, 130, Santhome High Road, Chennai-600028. The Registration Number MM - 557*Babylonia spirata* (Linnaeus, 1758).

Sample preparation

The collected snails were brought to the laboratory, the shells were broken and the soft body were separated and stored at -20° C until used.

Preparation of protein hydrolysate

The proteolytic digestion of *Babylonia spirata* was performed according to the method described by Je *et al*.,(2007). To produce peptides from tissue of *Babylonia spirata*, enzymatic hydrolysis was carried out with the enzyme Trypsin. The enzyme trypsin in 0.1 M phosphate buffer under optimal condition ; pH-8, temperature at 37°C at the enzyme/substrate ratio of 1:250 (w/w). Tissue of *Babylonia spirata* was homogenized with blender and then thoroughly mixed with enzyme. The enzyme substrate mixture was incubated for a period of 6h with constant stirring at the end of the incubation period the content was heated in a water bath for 10 minutes at 100°C. This heating inactivates and stops the enzyme activity. Then the mixture was centrifuged for 15 minutes at the speed of 10000rpm. The supernatant obtained was the protein hydrolysate. The hydrolysates were lyophilized to get a powdered sample and were stored at -20° C.

Determination of protein concentration:

The concentrations of protein hydrolysate were estimated by the Bradford's method using Bovine Serum albumin as a standard (Bradford, 1976).

SDS PAGE analysis

The molecular weight of the protein hydrolysate was confirmed by the SDS PAGE analysis (Laemmli, 1970) with the molecular weight marker ranging from 205-45kDa.

ANTIMICROBIAL ACTIVITY

Microbial strains used

Antimicrobial activity of the protein hydrolysate of *Babylonia spirata* was determined against 4 bacterial strains and 3 fungal strains. The bacterial strains include Gram positive bacteria *Staphylococcus aureus*, Gram negative bacteria such as *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Hafnia alvei* and fungal strains such as *Aspergillus fumigatus*, *Candida albicans*, and *Penicillium notatum*. These pathogenic strains were obtained from King Institute of preventive medicine, Guindy, Chennai, Tamilnadu, India. The organisms were periodically subcultured and maintained in nutrient agar slant at 4°C.

ANTIBACTERIAL ACTIVITY

Agar Well Diffusion Method

Antibacterial activity of the protein hydrolysate of *Babylonia spirata* was determined by agar well diffusion method (Bauer *et al*., 1996)on Nutrient agarmedium. The Nutrient agar Medium was poured in to the petriplates. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Wells were made in Nutrient agar plates by using a sterile cork borer of 5mm and add 20µl of protein hydrolysate of *Babylonia spirata* different concentration: 1000µg/ml, 500 µg /ml, 250 µg/ml were used. Distilled water used as negative control and chloramphenicol (1000µg/ml) used as positive control.

The plates were incubated at 37°C for 24 hours. The diameters of the zone of inhibition were measured in millimeter by using antibiotic zone measuring scale.

ANTIFUNGAL ACTIVITY

Agar Well diffusion method

Antifungal activity of the protein hydrolysate of *Babylonia spirata* was determined by agar well diffusion method on Potato Dextrose Agar (PDA) medium, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension.

Wells were made in PDA plate using a sterile cork borer of 5mm and add 20µl of protein hydrolysate of *Babylonia spirata* [Different concentration: 1000µg/ml 500µg/ml, 250µg/ml, Distilled water (negative control), Amphotericin-B 1000µg/ml (positive control)] each samples were loaded in the well. The plates were incubated for 24 hours at 37°C. Then the zone of inhibition was measured in millimeter by using antibiotic zone measuring scale.

Statistical analysis

All analyses were carried out in triplicate, and results are reported as the mean ± standard deviation (SD). Significant differences were analyzed by one-way ANOVA. Differences at $p < 0.05$ were considered significant.

RESULT AND DISCUSSION

Determination of protein concentration

2.6 mg/ml amount of protein was quantified at 595nm and Molecular weight of protein hydrolysate was obtained in SDS PAGE analysis, ranging from 40-200kDa shown in Fig. 1.

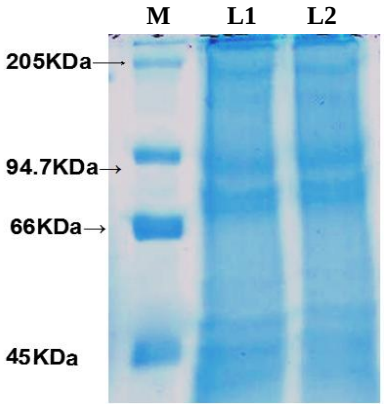
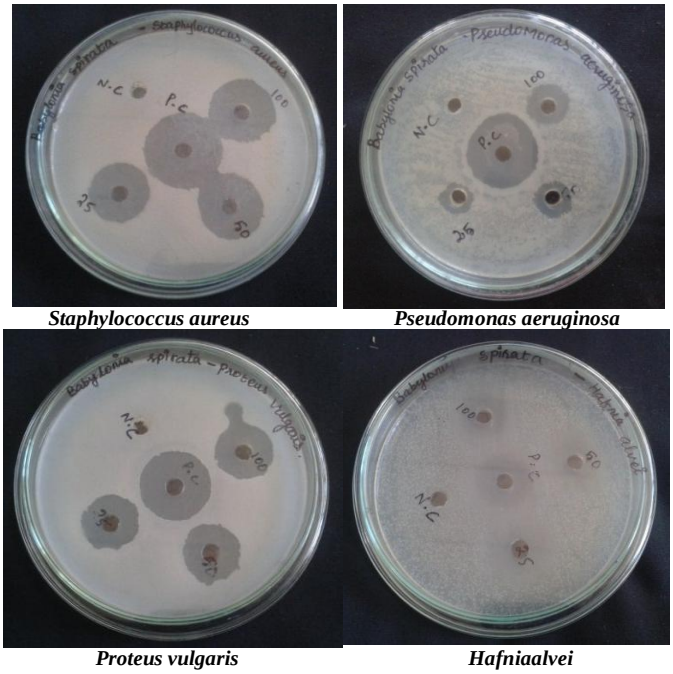


Fig. 1: SDS PAGE Analysis- *Babylonia spirata.*,
M- Molecular Weight marker High Range.,
L1 – Protein hydrolysate of *Babylonia spirata.*,
L2 – Protein hydrolysate of *Babylonia spirata*



Staphylococcus aureus ***Pseudomonas aeruginosa***

Proteus vulgaris ***Hafniaalvei***

Fig. 2: Antibacterial Activity - *Babylonia spirata.*

Table 1: Antibacterial Activity Of *Babylonia spirata*.

S. No.	Microorganisms	Zone of Inhibition in mm				
		1000µg/ml	500 µg/ml	250 µg/ml	Positive control 1000µg/ml	Negative control
1	*Staphylococcus aureus*	22.16 ± 1.04	19.9±0.90	18.6±1.44	24.8±0.76	-
2	*Pseudomonas aeruginosa*	11.5±0.5	7.83±2.8	7.16±2.8	23.7±0.76	-
3	*Proteus vulgaris*	16.6±0.57	13.6±0.57	10.5±0.5	20±0.5	-
4	*Hafniaalvei*	16±1	13.56±0.5	12.9±0.1	18.76±0.25	-

Antibacterial activity of protein hydrolysate of *Babylonia spirata*. The data was expressed as mean of triplicates ±SD measurements.

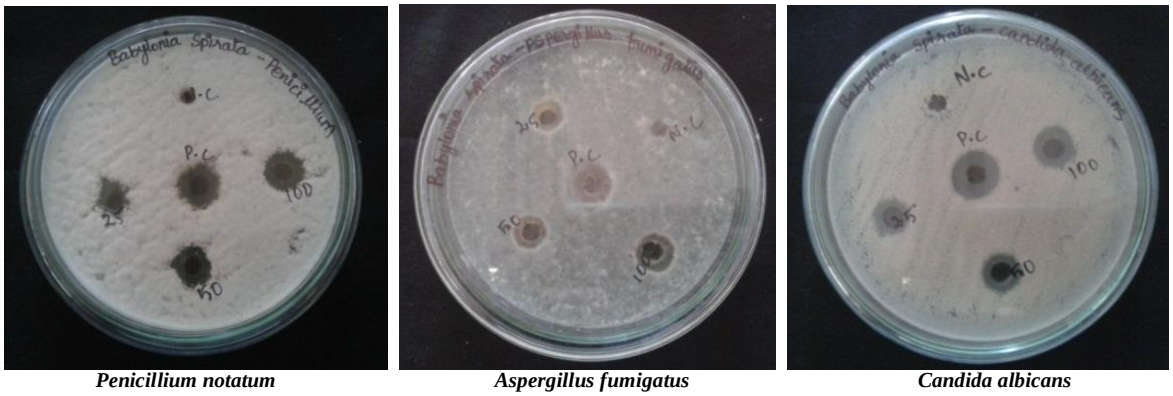
ANTIMICROBIAL ACTIVITY

Antibacterial activity

The antibacterial activity was performed on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Hafniaalvei* and the zone of inhibition of each organism were recorded and listed in Table 1 and Fig.2.

Antifungal activity

The antifungal activity was performed by using test organisms such as *Aspergillus fumigatus*, *Penicillium notatum*, *Candida albicans* and the zone of inhibition of each organism were recorded and listed in Table 2 and Fig.3.



Penicillium notatum ***Aspergillus fumigatus*** ***Candida albicans***

Fig. 3: Antifungal activity - *Babylonia spirata*

Table 2: Antifungal Activity Of *Babylonia spirata*.

S. No.	Microorganisms	Zone of Inhibition in mm				
		1000µg/ml	500 µg/ml	250µg/ml	Positive control 1000µg/ml	Negative control
1	*Penicillium notatum*	11.53+ 0.50	9.83+1.04	5.6+0.52	15+1	-
2	*Aspergillus fumigatus*	13.5+0.5	11.83+2.8	9.16+2.8	15.3+0.57	-
3	*Candida albicans*	10.5+0.5	8.56+ 0.60	7.2 + 1.2	13.6+0.57	-

Antifungal activity of protein hydrolysate of *Babylonia spirata*. The data was expressed as mean of triplicates +SD measurements.

DISCUSSION

Molluscs are widely used in world research institution for various studies, but recently they have been recognized as potential sources of antibacterial and antifungal properties. The overall objective of the current study for the capability of antibacterial and antifungal activity of enzyme digested protein hydrolysate of *Babylonia spirata.* The molecular weight of crude protein from *Babylonia spirata* was ranged from 2-110kDa on SDS PAGE (Periyasamy *et al*., 2012). In result of the present study clearly showed that, 2.6mg/ml protein concentrations were obtained in digested protein hydrolysate. Molecular weight ranging from 40 to 200kDa was found in the protein hydrolysate of *Babylonia spirata* which act as bioactive compounds for various biological activities. Antibacterial and antiviral activities have been previously described in the hemolymph of several molluscan species such as, sea hares, sea slung, oysters, and mussels (Mitta *et al*., 1999; Nakamura *et al*., 1988; Zasloff, 2002; Gueguen *et al*., 2006; Maktoob and Ronald, 1997; Olicard *et al*., 2005; Roch *et al*., 2008). The maximum zone was observed against *Staphylococcus aureus* 22.16 $\pm$1.04 mm at 1000µg/ml and the maximum zone was observed in *Aspergillus fumigatus*13.5$\pm$0.5 in 1000µg/ml concentration. Similar findings were reported in frog skin (Qian *et al*., 2008). The antibacterial activities of ethanol extracts of *Babylonia spirata* was observed maximum activity against *E.coli*, *K.pneumoniae*, *P.vulgaris* and *S.typhi* (prem *et al*., 1997). As an early report has been made, the crude ethanol extracts of *Babylonia spirata* showed good activities against *Pseudomonas aeruginosa* (Periyasamy *et al*., 2012).In the present study indicated that protein hydrolysate *Babylonia spirata* has many potential antibiotics. Marine molluscs have been found to produce a great diversity of novel bioactive compounds and to be a potential source for new drug discovery. There has been a remarkable progress in the prevention; control and even eradication of infectious diseases with improved hygiene and development of antimicrobial compounds.

CONCLUSION

The present study revealed that the protein hydrolysate of *Babylonia spirata* showed a potent antibacterial, and antifungal activity against pathogenic microorganisms. This investigation was followed by the screening tactics in investigation of novel bioactive compounds. It is promising that the tested gastropod synthesis novel antibiotics for bacterial infections. Further investigations intending to purify these active compounds should be considered to clarify their chemical composition.

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