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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF GASTROPOD, *HARPA DAVIDIS*, RODING 1798, FROM KANYAKUMARI COAST AGAINST ISOLATED HUMAN AND FISH PATHOGENS

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ABSTRACT

Objective: To screen the antibacterial drugs from different solvent extracts of tissue of marine gastropod *Harpa davidis* against clinically isolated human pathogenic bacteria and fish pathogens and to evaluate the biochemical composition and functional groups of the sample.

Methods: Crude extract of gastropod was tested for inhibition of bacterial growth. Antibacterial assay was performed by disc diffusion method. Biochemical composition of the tissue extract was analyzed using standard procedures. Moreover, Fourier transform infrared spectroscopy (FTIR) spectrophotometry analysis was also studied.

Results: The antimicrobial activity was measured based on the zone of inhibition around the disc impregnated with gastropod extract. The maximum zone of inhibition (10 mm) was observed against human pathogen *Bacillus cereus* in the crude methanol extract of *H. davidis* and the minimum zone of inhibition (2 mm) was noticed against *Shigella dysentriae* in the crude ethanol extract of *H. davidis*. In the case of fish pathogens, a maximum zone of (5 mm) was observed by methanol extract against *Vibrio anguillarum* and a minimum zone of 2 mm was observed by ethanol extract against *V. alginolyticus*. The proximate composition, such as protein, carbohydrate, and lipid contents of *H. davidis* tissue, was estimated. The results of this study revealed that the protein composition was high 38.42% followed by carbohydrate 9.61% and lipid 3.14%. FTIR spectrum of the sample *H. davidis* showed that 6 major peaks were shown at 3560.35, 2925.81, 2854.45, 1554.52, 1413.72, and 667.32/cm showing the presence of carboxyl, alkanes, aromatic, and alkyl functional groups.

Conclusion: The present study indicated that the marine mollusc collected from the Kanyakumari coast possessed several bioactive compounds of therapeutic interest. Further studies are going onto isolate specific compounds and to study the biological activities present in them.

Keywords: *Harpa davidis*, Human pathogens, Fish pathogens, Fourier transform infrared spectroscopy.

INTRODUCTION

The marine environment is a huge source to discover bioactive natural products. A wide variety of bioactive substances are being isolated and characterized from the food that is derived from the marine environment, several with great promise for the treatment of human and fish diseases. For the past two decades, the pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants. However, the advent of multiple resistant mechanism has limited the use of many major classes of antimicrobial compounds. The demand for effective and non-toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections. There is a vital interest in discovering new antimicrobial compounds with fewer environmental and toxicological risks and no resistance developed by the pathogens [1]. In marine invertebrates so far approximately 7000 marine natural products have been reported, 33% from sponges, 18% from coelenterates (sea whips, sea fans and soft corals), and 24% from representatives of other invertebrate phyla such as ascidians (also called tunicates), opisthobranch molluscs (nudibranchs, sea hares, etc.), echinoderms (starfish, sea cucumbers, etc.), and bryozoans (moss animals). In India, until today, 5070 species of Mollusca have been recorded, among which 3370 species are from marine environment [2], while rest from the fresh water and terrestrial environment.

Marine invertebrates offer a source of potential antimicrobial drugs [3]. Antimicrobial peptides are important in the first line of the host defense system of many animal species. Studies of antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in marine molluscs. Molluscs, which are widely distributed throughout the world, have many representatives in the marine and estuarine ecosystem. Many bioactive compounds have been investigated predominantly for their antimicrobial, cytotoxic, anti-tumor and anti-inflammatory, antileukemic, antineoplastic, and antiviral properties of molluscs [4-7].

Aquaculture has been the world's fastest growing food production system for the past decade [8,9]. In 1997, over 30% of food consumed by humans was provided by aquaculture [10]. In Asia, shrimp viral diseases caused farmers about 1 billion-dollar loss every year since 1994 [11]. The disease was initially controlled almost exclusively by the use of antimicrobial drugs. The increasing intensity of shrimp farming was inevitably paralleled by the increasing incidence of diseases in the farming systems. Pathogens such as *Vibrios* are the members of the normal bacterial flora of shrimps and act as primary and secondary invaders of shrimps in the culture system [12]. In the past 20 years, the pharmacological industry has been relatively successful in containing problems due to single resistance determinants. However, the advent of multiple resistance mechanism has severely reduced the use of currently used antibiotics.

The drug resistance in microorganisms is one of the serious problems all over the countries. The microorganisms develop multidrug resistance by their peculiar pattern of adaptation behavior and problems of multi-drug resistance in microorganisms are common in every field [13]. However, new drug classes with a novel mechanism of action will create effective therapy, at last, for a period of time [14]. Hence, there is an

urgent need for the discovery of new and novel antimicrobial drugs to effectively eradicate the diseases producing microorganisms. Therefore, the aim of the present study was to evaluate the antimicrobial activity of the tissue extracts of gastropod against different pathogenic bacterial strains.

METHODS

Specimen collection and identification

Live specimens of Gastropod, *Harpa davidis* were collected from, Kanyakumari coast of India. The collected fresh mollusc was preserved with ice and transported to the laboratory and identified by the standard literature [15]. The samples were washed with tap water until the sand and mud were removed from the shells. After that, the shells were broken using a hammer to remove the soft body tissue.

Preparation of extract

The freshly collected mollusc tissues each 25 g in wet weight were soaked in methanol, acetone and ethanol separately, and maintained for 3 days. The extracts were filtered through Whatman® No.1 filter paper and the solvents were concentrated by a rotary evaporator (VC100A Lark Rotavapor® at 30°C) with reduced pressure to give a dark brown gummy mass. The resultant residues were stored at 4°C for further analysis [16].

Bacterial cultures

Five species of human pathogens; *Bacillus cereus, Proteus vulgaris, Shigella dysenteriae, Streptococcus mutants*, and *Salmonella paratyphi* were obtained from the Christian Medical College Hospital, Vellore. Five fish pathogens: *Vibrio harveyi, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio alginolyticus*, and *Vibrio anguillarum* were obtained from Fisheries Department, Cochin.

Antibacterial assay

Antibacterial activity was performed using standard disc diffusion method [17]. The test cultures (bacteria 10^8 CFU/ml) were swabbed on top of the solidified media and allowed to dry for 10 minutes. The extracts of molluscs were applied onto 6 mm sterile discs in aliquots of 30 µL of solvent, allowed to dry at room temperature, and extract loaded discs were placed on agar plates seeded with isolated microorganisms and incubated at 37°C for 24 hrs. The susceptibility of the test organisms was determined by the radius of the inhibition zone around each disc.

Biochemical composition

Estimation of total protein

The Folin-Ciocalteu phenol method [18] was adopted for the estimation of total protein in the tissue.

Estimation of total carbohydrate

The total carbohydrate content was estimated using the procedure with phenol-sulfuric acid [19].

Estimation of total lipid

The chloroform-methanol extraction procedure was used for extracting lipid from the tissue [20].

Fourier transform infrared spectroscopy (FTIR) spectral analysis

The powdered sample *H. davidis* (10 mg) was mixed with 100 mg of dried potassium bromide and compressed to prepare as a salt disc. The disc was then allow to read spectrophotometerically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in the sample were analyzed.

RESULTS

Antibacterial screening

The antimicrobial activity of crude tissue sample of gastropod *H. davidis* was evaluated with human pathogens and fish pathogens using solvents such as ethanol, methanol and acetone at the concentrations of 30 µl by disc diffusion method. The crude methanol extracts were more active exhibiting broad spectrum of antimicrobial activity than the crude ethanol and acetone extracts. The maximum zone of inhibition 10 mm was observed for bacteria *B. cereus* in the crude methanol extract followed by 8 mm against *S. mutants.* Minimum zone of 2 mm was obtained by ethanol extract against *Shigella dysentriae* (Fig. 1). In the case of fish pathogens, a maximum zone of 5 mm was observed by methanol extract against *V. anguillarum* and a minimum zone of 2 mm was observed by ethanol extract against *V. alginolyticus* and by acetone and methanol extract against *V. parahaemolyticus* and *V. harveyi* (Fig. 2).

Biochemical composition

The proximate composition such as protein, carbohydrate, and lipid contents of *H. davidis* tissue was estimated. The results of the present study revealed that the protein composition was high 38.42% followed by carbohydrate 9.61% and lipid 3.14%.

FTIR spectrophotometry (FTIR spectrum analysis)

FTIR spectrum of the sample *H. davidis* showed that 6 major peaks were shown at, 3560.35, 2925.81, 2854.45, 1554.52, 1413.72, 667.32/cm showing the presence of carboxyl, alkanes, aromatic and alkyl functional groups, whereas all the remaining peaks were very close and at 3442.70, 3417.63, 2925.8, 2854.45 and 1195.78/cm (Fig. 3).

DISCUSSION

In recent years, great attention has been paid to the bioactivity of natural products because of their potential pharmacological utilization. Most of the homeopathic medicines are either of plant or animal origin. Thousands of bioactive compounds identified in marine

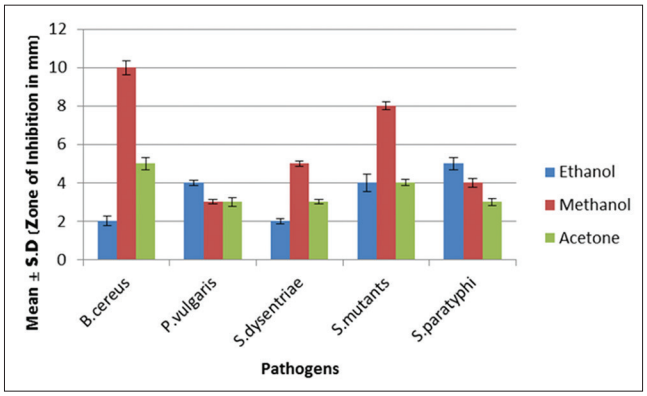

Fig. 1: Antibacterial activity of tissue extract of *Harpa davidis* against human pathogens

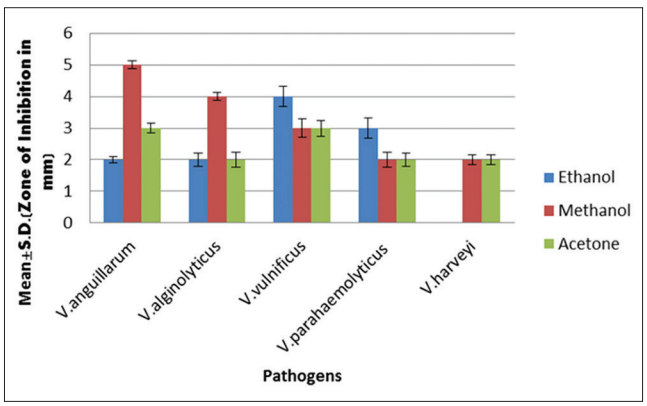

Fig. 2: Antibacterial activity of tissue extract of *Harpa davidis* against fish pathogens

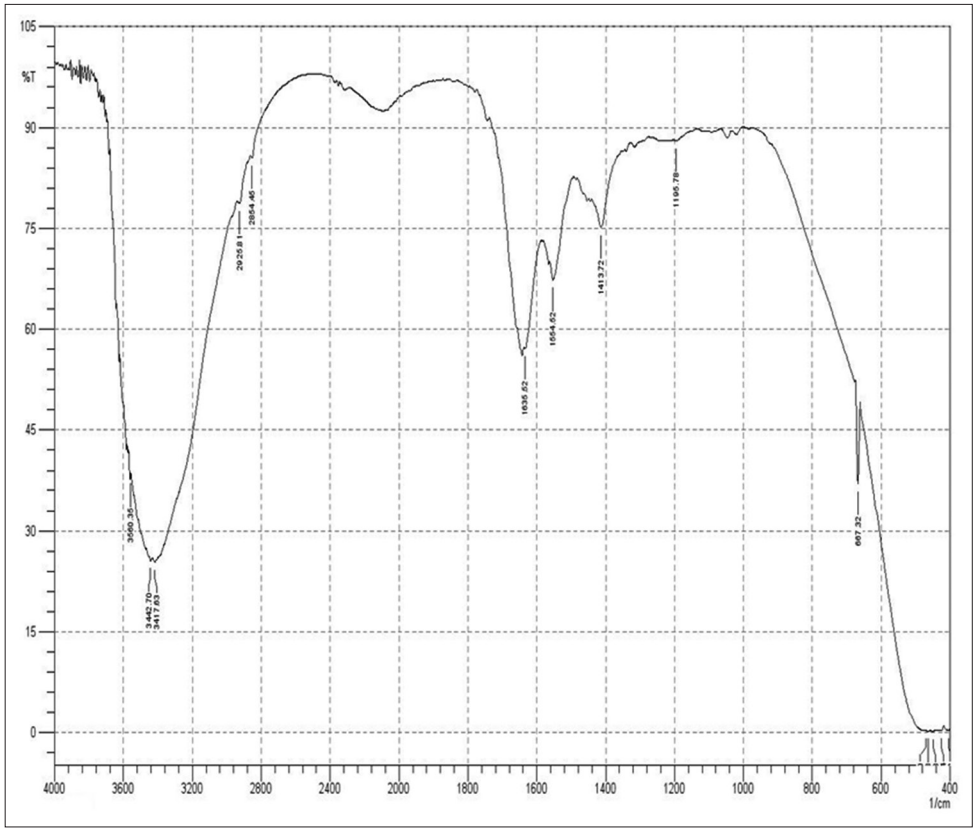

Fig. 3: Fourier transform infrared spectrum of the sample of *Harpa davidis*

organisms reveal that sea creatures constitute a large reservoir for pharmacologically active drug recently reviewed [21].

In the present investigation, solvent extracts of *H. davidis* was concentrated under reduced pressure to give a dark brown gummy mass of 2.62 g, respectively. In this study, a distinct antimicrobial activity has been observed against isolated bacterial strains. In this experiment, the solvents ethanol, methanol and acetone, were used for extraction. The molluscs crude extracts were assayed against isolated human and fish pathogenic microbes. From the antimicrobial assay ethanol, methanol, and acetone extracts showed promising sensitivity against human and fish pathogenic bacteria. From the human pathogenic bacteria tested, showed more sensitivity (10 mm) against methanol extracts of *H. davidis* and from fish bacterial pathogenic microbes tested, *V. anguillarum* showed most sensitivity (5 mm), against methanol extract. These findings are consistent with previous studies on molluscs. Molluscan extracts produced the largest zones of inhibition out of all the marine extracts that were tested against *Escherichia coli* and *Penicillium atrovenetum*. Significantly, different species of mollusc were priority listed for activity in the different assays. As an early report has been made, the crude ethanol extracts of *Babylonia spirata* showed good activities against *Pseudomonas aeruginosa* [22]. The methanol extract of *Hemifusus pugilinus* possessed the highest activity against *E. coli*, and the lowest activity was observed against *Klebsiella oxytoca* [23]. The methanolic extract of *Chicoreus virgineus* and *Chicoreus ramous* experimentally analyzed and observed the broad spectrum antimicrobial activities of body tissue extract [24]. The antibacterial activities of ethanol extracts of gastropod *B. spirata* and *Turbo brunneus* were observed maximum activities against *E. coli*, *Klebsiella pneumoniae*, *P. vulgaris* and *Salmonella Typhi*, respectively [25]. The present work reveals that gastropod *H. davidis* has potential antimicrobial activity.

Foods from the sea have for hundreds of years been a source of high-quality protein. In the five basic groups seafood belongs to the same category as meat, poultry, eggs, dried beans and peas-all major sources of protein. Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body [26].

The present investigation revealed that the maximum level of protein content in *H. davidis* body tissue was 38.42%. Babu *et al*., 2010 [27] assessed the percentage of protein which was ranged from 19.25% to 27.9% in the Mesogastropod, *Bursa spinosa*. The protein content was varied from 18.71% to 29.81% reported by Shanmugam *et al.,* [28]. Arularasan [29] contributed the percentage of protein ranged from 47.86% to 70.18% males and from 49.64% to 72.21 in females *Strombus canarium* gastropoda (Gulf of Mannar).

Carbohydrates are a group of organic compounds including sugars, starches and fiber, which is a major source of energy for animals. In the present study, the percentage of carbohydrates in the body tissue was 9.61%. Arularasan [29] estimated maximum levels (5.31%) of carbohydrate in *Littorina quadricentus* and (4.69%) in *Nodilittorina pyramidalis*. Thivakaran [30] reported the carbohydrate in *Pythia plicata* values from 0.84 % to 3.04%. The carbohydrate content of the present study was higher when compared to the previous studies.

The lipids are highly efficient as a source of energy, in that they contain more than twice the energy of carbohydrate and proteins. In the present study, lipid content of tissue was 3.14%. Shanmugam [31] reported lipid content in *Rapana rapiformis* which ranged from 0.85 to 2.12% in male and 0.95-2.96% in female gastropod. Rajkumar [32] found the highest level of lipid 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium*, respectively.

The previous study of FTIR Showed 8 major peaks in lyophilized sample of *B. spirata* [33]. In the present study, the results of FTIR analysis reveals the presence of bioactive compound signals at different range (665.44-3996.51/cm). Molluscs are widely used in world research institution for various studies, but only recently they have

been recognized as potential sources of antibacterial and antifungal substances. The potential of marine mollusc as a source of biologically active products is largely unexplored. Hence, a broad-based screening of marine molluscs for bioactive compounds is necessary. However, by far the majority of marine organisms are yet to be screened, and the potential for discovering a useful antibiotic is sufficient to affirm further research.

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