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Research Article
Identification of Bioactive Peptides in Mussel Species of Kanyakumari Coast

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Abstract

Background and Objective: Anti-bacterial activity was evaluated in the whole body crude extracts of the 3 edible marine bivalves, namely *Perna perna* (brown mussel), *Perna viridis* (green mussel) and the parrot mussel collected from the coastal villages of Kanyakumari district. **Materials and Methods:** Six different solvents, namely ethanol, methanol, acetone, ethyl acetate, hexane and butanol were used to prepare the extracts. The efficacies of the whole body extract of different solvents were assessed for the antimicrobial activity against 13 different pathogenic bacteria viz., *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Staphylococcus epidermis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella paratyphi*, *Streptococcus pyogenes*, *Vibrio* sp., *Shigella* sp. and *Haemophilus influenza*. **Results:** Of the 6 different solvents methanol exhibits the highest activity followed by ethanol and acetone. The methanolic extracts of *P. viridis* exhibit the highest activity against *E. coli*, *S. typhi* and *P. aeruginosa*. The ethanolic extract of *P. viridis* shows the highest activity against *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae* and acetone extract showed good activity against *E. coli*. The methanolic extracts of *P. perna* exhibit the highest activity against *E. coli*, *S. aureus*, *S. typhi*, *P. vulgaris*. The ethanolic extract of *P. perna* is active against *P. vulgaris* and *B. subtilis* and the ethanolic extract showed good activity against *P. aeruginosa* and *Shigella* species. The methanolic extracts of the parrot mussel exhibit the highest activity against *E. coli* and *Streptococcus pyogenes*, ethanol extract shows good activity against *E. coli* and *Staphylococcus aureus* and the acetone extract shows maximum antibacterial activity against *P. aeruginosa*. **Conclusion:** The findings of the present study confirm that the mussel species have antimicrobial activity and that this activity appears to be dependent on the solvent used for the extraction process. FTIR analysis reveals the presence of bioactive compounds signals at different ranges.

Key words: Mussel, antibacterial activity, pathogens, bioactive peptides, FTIR

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to the alarming rise in the occurrence of antibiotic-resistant bacterial strains, the identification of new antimicrobial compounds has become one of the frontier areas in biomedical research. Marine invertebrates are known to rely on innate immune mechanisms which include both interacting cellular and humoral components to protect themselves against potential pathogen[1]. The innate immune mechanism in marine invertebrates is fairly well-known, as defense mechanism against potential pathogens. Moreover, it has been well known that the innate immunity is triggered immediately after microbial infection to produce antimicrobial compounds including small antimicrobial peptides (AMP). In recent years, it has widely been recognized that AMPs are strong defensive weapons against bacteria and/or fungi, viruses, or parasites in multicellular organisms[2]. Furthermore, AMPs are also known as major components of the innate immune defense system in invertebrates[3].

According to the World Health Organization[4] resistance of diseases-causing organisms to antibiotics is an emerging danger. The emergence of antibiotic resistance by bacteria has become a medical catastrophe and we maybe entering a 'post-antibiotic' era where antibiotics are no longer effective. Development of new microbial compounds to combat the resistant organisms is becoming critically important[4]. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often adulterated and with side effects. Therefore, there is a need to search for new infection-combating strategies to control microbial infections[5]. Pharmaceutical industries are according importance to the compounds derived both from traditional sources (soil and plants) and non-traditional sources like marine organisms[6]. Interest in the importance of marine organisms as sources of new substances is growing. With marine species comprising approximately half of the total global biodiversity, the sea offers an enormous resource for novel compounds[7].

Bacterial infection causes the highest rate of mortality in human populations and aquaculture organisms[8]. For example, *Bacillus* species is responsible for causing food-borne diseases[9]. *Staphylococcus aureus* is the causative agent of inflammatory bowel disease[10]. *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenza* and *Staphylococcus epidermis* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella* sp, *Shigella* species and *Vibrio* species cause diarrhea and typhoid fever[11]. *Pseudomonas aeruginosa* is an important and much prevalent pathogen among burn patients and it is capable of causing life-threatening illness[8]. The revolutionary therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to the changing patterns of resistance in pathogens and has side effects. These limitations demand improved pharmacokinetic properties, which necessitates continued search for new antimicrobial compounds for the development of drugs[12]. Hence, the interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during recent years[13].

The rich diversity of marine organisms offers a great scope for the discovery of new bioactive substances. Thus the marine environment is an exceptionally vast reservoir of natural bioactive products, many of which exhibit structural features that are not found in terrestrial natural products[14]. Due to the delicate taste and the high nutritional value (high content of proteins, vitamins and trace elements), molluscan bivalves are very valuable foodstuffs[15,16]. In India, 2 species of mussels are found, the Asian green mussel, *P. viridis* and the brown mussel *P. indica*, of which the brown mussel is more prevalent. The brown mussel was named earlier as *Perna indica*[17] but recently it is identified as *Perna perna*[18]. The brown mussel had a wider distribution all along the Kanyakumari coast; the green mussel has very restricted distribution along the Kanyakumari coasts of Enayam, Colachel and Kadiyapattinam. In these localities, yet another morphotype of mussel, with light yellowish green shell colouration is found, which locally known as the parrot mussell[19]. Padi[20] also reported the existence of intermediate morphotypes of mussels from the Indian coast. Based on a study using molecular tools Divya *et al.*[19] reported that the parrot mussel is only a morphotype of the brown mussel and not a true hybrid of *P. viridis* and *P. perna*. Many researchers[21-23] have reported that mollusks have high antimicrobial activity, however only limited studies have been made on the antimicrobial potential of mussel species.

From 1960's-1990's approximately 300 bioactive marine natural products have been filed for patent. Approximately 6,500 bioactive compounds have been isolated from marine organisms[24]. Among the invertebrates, the mollusks are highly delicious seafood because of their nutritive value next to finfishes and crustaceans. They are also very good sources of bio-medically important products[25]. Against the background of these pathological and clinical findings a new investigative interest has emerged to look for novel and persuasive antibacterial peptides from mussels which are the ultimate of therapeutic use. Among the mollusks, mussels are very good source of bioactive compounds[26]. Considering on one hand the fact that the marine animals can survive in a hostile environment where they are surrounded by various

pathogenic organisms, including human pathogens[27] and the fact that they are potential sources of bioactive compounds and considering on the other the importance of the group vis-à-vis the paucity of information in this field, an attempt is made in the present study to evaluate the antimicrobial activity in the 3 commonly occurring edible bivalve mussels, namely *Perna indica* (brown mussel), *Perna viridis* (green mussel) and the parrot mussel. In continuation of the same effort, an attempt is also made to assess the functional group responsible for the antimicrobial activities by FTIR analysis.

MATERIALS AND METHODS

Sample collection and processing: Mussels namely the green mussel (*Perna viridis*), the brown mussel (*Perna indica*) and the parrot mussel (Fig. 1) were examined. A total of fifty individuals were analyzed for the presence of antibacterial substances. The mussels were collected mainly from rocky beaches of Kanyakumari district during November, 2017 and were wrapped in seawater-wetted newspaper and placed in plastic buckets and transported to the laboratory for analysis. The 3 types of mussels were separated, washed and kept at room temperature. Then the shells were opened with a stainless steel knife, the meat was removed from the shells after eliminating the byssus thread and rinsed in tap water. Each type of mussel meat was dried in a hot air oven at 40°C for 3 days. The dried meat samples were powdered using an electrical blender.

Extraction: About 25g of the powdered mussel samples were subjected to solvent extraction using 6 different solvents in a Soxhlet apparatus following the standard procedure[28]. The samples were loaded into the inner tube of the Soxhlet apparatus and then fitted into a round-bottomed flask containing the solvents. The solvent was boiled gently (40°C) over a heating mantle using the adjustable rheostat. The procedure was continued until complete extraction was effected (8 h). It was then filtered using a Whatman No. 1 filter paper. The filtrate was evaporated and dried at 55-60°C to yield a residue of methanol, 95% ethanol, acetone, ethyl acetate, hexane and butanol for antibacterial activity. The advantage of this procedure is that fresh solvent can continually extract the samples more effectively with minimum solvent.

Antibacterial activity: Thirteen human pathogens namely *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Staphylococcus epidermis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella paratyphi*, *Streptococcus pyogenes*, *Vibrios.*, *Shigella* sp. and *Haemophilus influenza* were obtained from the Christian Medical College Hospital, Vellore. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Antibacterial activity of the prepared crude extracts was analyzed using well-cut diffusion technique[29]. The wells with 0.7 cm sterile cork borer were punched on to Muller Hinton Agar plates that were previously swabbed with the bacterial cultures. The wells were filled with

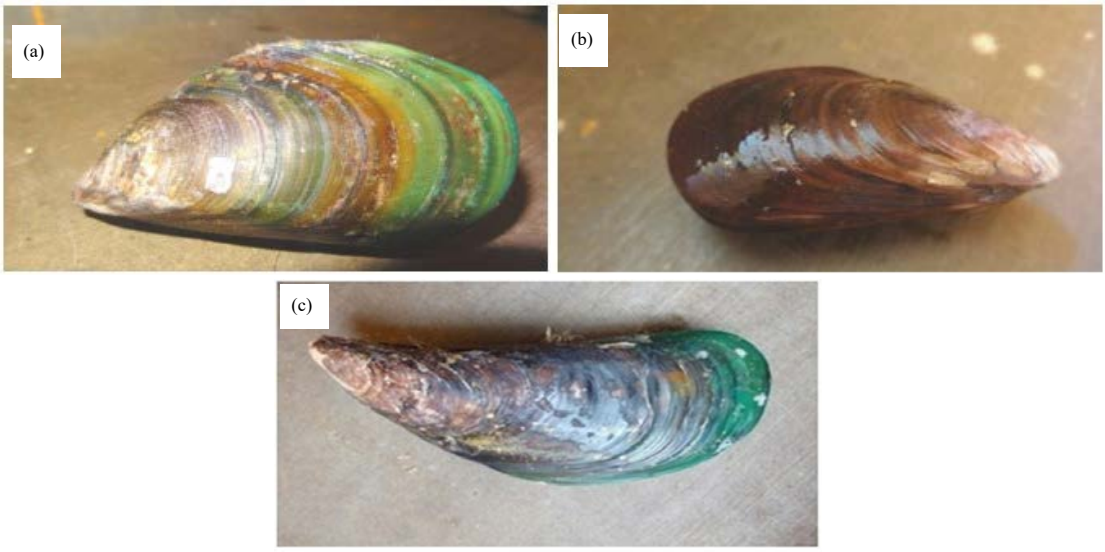


Fig. 1(a-c): (a) Green mussel (*P. viridis*), (b) Brown mussel (*P. perna*) and (c) Parrot mussel species of Kanyakumari coast

50 µL of different solvent extracts. About 10% of DMSO was used as a negative control. Streptomycin sulphate was used as the positive control. To prevent drying, the plates were covered with sterile plastic bags. All plates were later incubated at 37°C for 24 h[30]. The antibacterial activity was measured based on the inhibition zone around the well with mussels extract. The result was obtained by measuring the inhibition zone for each well and expressed in millimeter.

Fourier transforms infrared spectroscopy (FTIR) spectral analysis: The methanolic extracts of the three mussels were analyzed qualitatively for the active compounds by Fourier Transform Infra-Red (FTIR) spectroscopic method as described by Kemp[31]. Approximately 5 mg of sample was mixed with 100 mg of dried potassium bromide (KBr) and subjected to a pressure of 5×10^6 pa and made into a clear pellet of 3 mm diameter and 1 mm thickness. Absorbance spectra were recorded using FTIR Spectrometer equipped with a KBr beam splitter and an air-cooled DTGS detector, Department of Chemistry, Karunya University. The absorption of light intensity of the peak was calculated using the baseline method. The frequencies for all sharp bands were accurate to 0.01 cm^{-1}.

Statistical analysis: All analysis were carried out in triplicate and results are reported as the Mean±Standard deviation (SD).

RESULTS

Antimicrobial activity of mussel species using different solvents: The antibacterial activities of *P. viridis*, *P. perna* and the parrot mussel are respectively presented in Table 1-3. The results of the present study may indicate the presence of

Table 1: Antibacterial activity of *P. viridis* against pathogen

	Zone of inhibition (mm)						
Pathogens	Methanol	Ethanol	Acetone	Ethyl acetate	Hexane	Butanol	P-control
Salmonella typhi	9±0.30	9±0.25	6±0.35	4±0.47	2±1	1±0.12	6±0.52
Escherichia coli	11±0.3	13±1.2	9±0.14	4±0.5	1±0.54	1±0.42	8±0.36
Psudomonas aeruginosa	90±0.4	14±1.4	8±0.25	4±0.3	3±0.12	3±0.75	7±0.52
Staphylococcus aureus	3±0.5	3±0.78	1±0.4	2±0.6	1±0.42	1±0.52	7±0.63
Proteus vulgaris	6±0.51	5±0.25	2±1.1	2±0.52	3±0.25	1±0.47	8±0.52
Staphylococcus epidermis	2±0.31	1±0.14	1±0.23	1±0.15	1±0.27	2±1.02	7±0.60
Klebsiella pneumonia	8±0.14	9±0.17	3±0.14	1±0.12	1±0.42	1±0.1	5±0.5
Bacillus subtilis	20±0.2	2±0.36	2±0.18	1±0.18	1±0.41	1±0.25	6±0.4
Salmonella paratyphi	3±0.51	1±0.54	3±0.47	1±0.19	1±0.52	1±0.36	6±2.3
Streptococcus pyogens	6±0.31	3±0.2	3±0.28	2±0.14	1±0.63	1±0.21	6±2.6
Vibrio sp.	4±0.42	4±0.5	1±0.14	3±1.2	2±0.53	1±0.24	6±1.2
Shigella sp.	2±0.14	3±0.5	5±0.17	2±1.4	2±0.47	3±0.36	6±1.8
Haemophillus influenza	3±0.47	2±0.5	1±0.25	1±1.25	1±0.53	1±0.42	6±1.5

Data was expressed as mean of triplicates±SD measurements

Table 2: Antibacterial activity of *Perna perna* against pathogen

	Zone of inhibition (mm)						
Pathogens	Methanol	Ethanol	Acetone	Ethyl acetate	Hexane	Butanol	P-control
Salmonella typhi	9±1.02	7±0.25	6±0.2	7±0.25	2±0.39	1±0.25	6±0.56
Escherichia coli	10±1.0	5±0.14	4±1.3	7±0.18	2±0.11	1±0.36	6±0.41
Psudomonas aeruginosa	3±0.25	4±0.36	8±1.4	2±0.36	1±0.4	1±0.48	6±0.71
Staphylococcus aureus	10±1.2	9±0.47	7±1.8	6±0.65	2±0.17	2±0.47	7±0.85
Proteus vulgaris	9±0.5	10±0.5	4±1.1	5±0.87	2±0.36	3±0.68	7±0.88
Staphylococcus epidermis	2±1	2±0.28	1±0.25	1±0.12	1±0.28	1±0.17	6±0.56
Klebsiella pneumonia	2±2.2	6±0.48	2±0.78	2±0.36	1±0.39	1±0.35	5±0.28
Bacillus subtilis	4±1.2	10±0.5	5±0.69	5±0.98	2±0.78	2±0.5	5±0.24
Salmonella paratyphi	2±1.6	2±0.3	2±0.28	1±0.7	1±0.54	1±0.41	6±0.48
Streptococcus pyogens	3±0.6	2±1.1	3±0.17	1±0.4	1±0.28	1±0.23	5±1.2
Vibrio sp.	6±0.25	6±1.6	5±0.24	6±2.1	2±0.45	1±0.28	6±2.3
Shigella sp.	5±1.2	5±0.25	8±0.75	4±1.36	2±0.78	2±0.22	6±2.8
Haemophillus influenza	2±0.3	2±0.39	3±0.69	1±0.78	1±0.29	1±0.12	6±2.56

Data was expressed as mean of triplicates±SD measurements

Table 3: Antibacterial activity of parrot mussel against pathogen

	Zone of inhibition (mm)						
Pathogens	Methanol	Ethanol	Acetone	Ethyl acetate	Hexane	Butanol	P-control
Salmonella typhi	8±0.40	6±0.47	2±0.12	4±0.57	1±0.36	2±0.27	8±0.47
Escherichia coli	11±1.20	10±0.40	7±0.17	7±0.59	6±1.50	1±0.25	9±0.38
Psudomonas aeruginosa	4±0.20	4±0.20	11±0.23	7±0.55	2±1.22	2±0.75	6±0.27
Staphylococcus aureus	8±0.36	10±0.27	3±0.58	6±0.63	2±0.25	1±0.74	8±0.75
Proteus vulgaris	4±0.25	6±0.36	1±0.17	1±0.23	1±0.36	1±0.83	8±0.85
Staphylococcus epidermis	2±0.32	-	2±0.56	2±0.25	-	-	5±0.94
Klebsiella pneumonia	2±0.52	6±0.75	2±0.87	2±0.47	1±0.47	1±0.54	5±0.41
Bacillus subtilis	7±0.64	6±0.85	5±0.39	1±0.28	1±0.47	2±0.33	6±0.53
Salmonella paratyphi	4±0.75	4±0.82	4±0.75	3±0.38	2±0.54	1±0.25	6±0.31
Streptococcus pyogens	10±0.50	3±0.91	8±0.51	6±0.14	1±0.50	2±0.27	5±0.37
Vibrio sp.	7±0.25	3±0.74	4±0.98	3±2.3	1±1.70	1±0.39	6±0.27
Shigella sp.	4±0.52	4±0.36	6±0.24	3±1.47	3±1.36	2±0.17	7±0.70
Haemophillus influenza	2±0.14	2±0.18	4±0.36	1±0.38	1±1.78	1±0.38	6±0.44

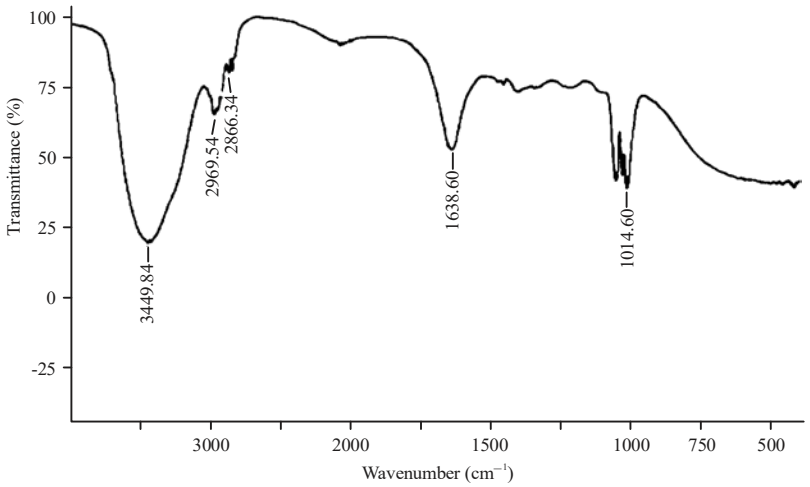
Data was expressed as mean of triplicates±SD measurements

different natural antibacterial substances in the tested mussels, which create the inhibition zones on plates against tested bacteria. The 3 mussels were screened for the antibacterial activity, all the species had superior activity against the microbial growth with different solvents. However, methanol and ethanol proved to be the best solvents and showed good antibacterial activities against pathogens. The highest zone of inhibition (14 mm) was recorded in ethanol extract of *Perna viridis* against *Pseudomonas aeruginosa*. The positive control streptomycin sulphate showed a zone of inhibition only about 7 mm against *Pseudomonas aeruginosa*. It is, therefore, clear that the susceptibility of *Pseudomonas aeruginosa* to the ethanol extract of mussel *P. viridis* was distinctly higher than that to the antibiotic (Table 1). This was followed by the methanol and acetone extracts which created a zone of inhibition of 9 and 8 mm respectively to the same pathogen. The methanol extract of *P. viridis* also found to be active against, *E. coli* (11 mm), *S. typhi* (9 mm), *K. pneumoniae* (8 mm) and *S. pyogenes* (6 mm). Moderate antibacterial activity was seen in the methanol and ethanol extracts against *Vibrio* species (4 mm) and acetone extract against *Shigella s*pecies (5 mm). Of the 6 solvents used in this study, hexane and butanol in extract of *P. viridis* showed less activity with their zones of inhibition of 1-3 mm when compared to the methanol, ethanol and acetone extracts. The ethyl acetate extract of *P. viridis* was able to inhibit the *S. typhi*, *E. coli* and *P. aeruginosa* which showed an inhibition range of 4 mm.

The methanol extract of *P. perna* brought about an inhibition zone of 10 mm against *E. coli* and *Staphylococcus aureus* but showed less activity (2 mm) against *Haemophilus influenza*, *S. paratyphi*, *Staphylococcus epidermis* and *Klebsiella pneumoniae*. The ethanol extract of *P. perna* showed the highest zone of inhibition of 10 mm against *Proteus vulgaris* and *Bacillus subtilis*. Moderate antibacterial activity (7 mm) was seen in acetone and ethyl acetate extracts of *P. perna* against *Staphylococcus aureus* and *S. typhi*, *E. coli*, respectively. Hexane and butanol extracts of *P. perna* were not so effectual in inhibiting the growth of pathogens for they created zones of inhibition only in the range of 1- 2 mm (Table 2).

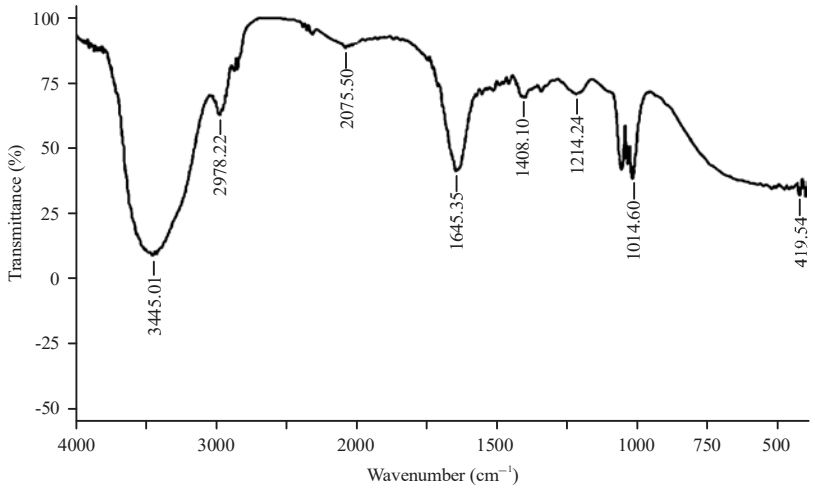
The methanol extract of the parrot mussel showed the highest activity of 11 mm against *E. coli*, 10 mm against *Streptococcus pyogenes*, 8 mm against *S. typhi* and 8 mm against *S. aureus*. It produced a zone of inhibition of 2 mm against *Staphylococcus epidermis*, *K. pneumoniae* and *Haemophilus influenza* (Table 3). Ethanol extract of the parrot mussel could inhibit the pathogens *E. coli* and *S. aureus* with the zones of inhibition of 10 mm. Both ethanol and methanol extracts of the parrot mussel showed bigger zones of inhibition than the positive control streptomycin sulphate. Ethanol, hexane and butanol extracts showed no activity against *Staphylococcus epidermis*. The hexane extract of the parrot mussel was active only against *E. coli* whereas the butanol extract fared poorly against all pathogens. DMSO, the negative control, did not show any inhibition of the tested pathogens.

In this study good antibacterial activity was observed for methanol and acetone extracts of the parrot mussel, ethanol and ethyl acetate extracts of *P. perna* were superior to the methanol, ethanol and acetone extracts of *P. viridis.* The hexane and butanol extracts of all the mussels showed less antibacterial activity when compared to the other extracts. This study concludes that the three mussel samples having antimicrobial compounds are responsible for antibacterial activity and that the levels of potential vary between the species and the solvent used for the extraction.



No.	Peak	Intensity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area
1	1014.6	38.99	15.54	1025.21	960.59	16.13	2.08
2	1638.6	52.89	0.23	1639.56	1527.69	18.29	-2.93
3	2866.34	80.25	4.05	2885.63	2852.84	2.86	0.4
4	2969.54	65.14	1.12	2974.36	2958.93	2.78	0.05
5	3449.84	19.63	0.14	3468.16	3447.91	14.08	0.04

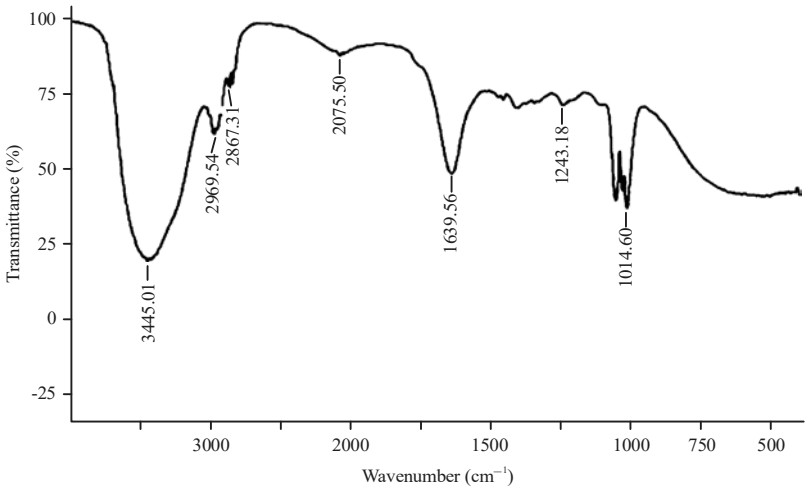
Fig. 2: FTIR spectra of the *Perna perna* fractionated sample



No.	Peak	Intensity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area
1	419.54	31.62	5.57	427.25	409.89	8.2	0.76
2	1014.6	37.98	15.7	1025.21	952.88	17.69	1.68
3	1214.24	70.48	5.75	1279.82	1158.3	16.53	2.22
4	1408.1	69.55	0.08	1409.06	1406.17	0.46	0.00
5	1645.35	40.93	1.56	1659.82	1642.46	6.19	0.1
6	2075.5	88.23	1.2	2107.32	2060.06	2.37	0.12
7	2978.22	62.53	1.09	3001.37	2974.36	5.08	0.09
8	3445.01	8.45	0.35	3447.91	3431.51	17.06	0.12

Fig. 3: FTIR spectra of the *Perna viridis* fractionated sample

FTIR spectral analysis: Fourier Transform-Infra Red spectroscopic analysis led to the identification of functional groups in the mussel species and KBr was used as the standard. The outcomes of the analyses are presented in Fig. 2-4. In the case of *P. perna*, from the graph plotted with wave number against intensity, 5 peaks with frequencies ranging from 1,014.60-3,449.84 cm^{-1} were recorded (Fig. 2). The functional groups viz. N-H stretch in the secondary amine, free NH appeared at 3449.84 cm^{-1}, C-H stretch group C-methyl appeared at 2,969.54 cm^{-1} and aldehyde -C(=O) H group



No.	Peak	Intensity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area
1	1014.6	37.19	14.33	1024.25	959.63	16.69	1.62
2	1243.18	71.49	2.54	1284.65	1215.21	9.37	0.39
3	1639.56	48.74	32.34	1879.71	1530.58	45.89	18.84
4	2075.5	87.95	1.26	2105.39	2061.99	2.25	0.11
5	2867.31	77.73	3.86	2884.67	2852.84	3.21	0.38
6	2969.54	61.81	0.78	2973.4	2957.97	3.13	0.03
7	3445.01	19.47	0.21	3447.91	3432.48	10.84	0.04

Fig. 4: FTIR spectra of the parrot mussel fractionated sample

appear at 2,866.34 cm^{-1} with the C-H stretch. The N-H stretching frequency in primary amide 2 appeared at 1,638.60 cm^{-1} and C-N stretching frequency in aliphatic amines group appeared at 1,014.60 cm^{-1}.

In the case of *P. viridis*, in the graph plotted with wave number against intensity, 8 peaks with frequencies ranging from 419.54-3,445.01 cm^{-1} were recorded. Here, NH stretch group primary amide (polypeptides) frequency appeared at 3,445.01 cm^{-1}, CH stretching C-methyl group frequency appeared at 2978.22 cm^{-1}, N=H stretching frequency in azide group appeared at 2,075.50 cm^{-1}, with the -N-H stretch in primary amide solid amide II appeared at 1,645.35 cm^{-1} (Fig. 3). The -N=N stretching azo group frequency appeared at 1,408.10 cm^{-1}, aliphatic amide (-C-N) frequency appeared at 1,214.24 cm^{-1} and the -C=O deformation frequency of primary amide (solid) Amide 2 appeared at 1,014.60 cm^{-1} and the Amide 3 group-C-N stretch at 419.54 cm^{-1}. The FTIR spectrum of *Perna viridis* confirms the presence of primary amide group, secondary amide, the aliphatic amine group, C-methyl and azo groups.

In the case of the parrot mussel, from the graph plotted with wave number against intensity, seven peaks with frequencies ranging from 1,014.60-3,445.01 cm^{-1} were recorded (Fig. 4). The N-H group primary amide, free NH frequency appeared at 3,445.01 cm^{-1} and C-H stretching C-methyl group appeared at 2,969.54 cm^{-1}. The C-H deformation frequency of -C(=O) H aldehyde group appeared at 2,867.31 cm^{-1} (Fig. 4). The isocyanate compound N=C=O group frequency appeared at 2,075.50 cm^{-1} and the N-H stretch on primary amide (solid) Amide 2 group appeared at 1,639.56 cm^{-1}. The N-H stretching secondary amide (Amide 3) group appeared at 1,243.18 cm^{-1} and C-N stretching frequency in aliphatic amine group appeared at 1,014.60 cm^{-1}.

DISCUSSION

In this study, a predominant antibacterial activity has been observed against some bacterial strains. A methanol extract of *Perna viridis* showed the highest activity against *E. coli*, *S. typhi* and *P. aeruginosa*, ethanol extracts showed the highest activity against *P. aeruginosa*, *E. coli*, *S. typhi* and other extracts showed the lowest activity against all the tested pathogens. Similarly, the methanol extract of *P. perna* exhibited the highest activity against *E. coli*, *S. aureus* and ethanol extract displayed the highest activity against *Proteus vulgaris* and *Bacillus subtilis*. Only acetone extract of *P. perna* showed the highest activity against *P. aeruginosa* and ethyl acetate extract showed a moderate activity against *E. coli* and *S. typhi*. Hexane and butanol extracts showed only 1-2 mm of inhibition zone against all the pathogens. The results of this study agree with those of the antimicrobial activity of the gill extraction of *Perna viridis*[32], the antimicrobial activities of the bivalve mollusk *Meretrix meretrix* and *Meretrix casta*[33], the antibacterial

activities of the green mussel (*Perna viridis*) and edible oysters, *Crassostrea madrasensis*, as reported by Annamalai *et al*.[26]. There has been no work done on the antimicrobial properties of the parrot mussel.

Some authors described the presence of trace amounts of antibiotics in mollusks[34-38]. As filter-feeding organisms, mollusks are exposed to high concentrations of bacteria, including pathogens. For this reason, their immune defense system must be based on non-specific, rapid cellular and humoral responses. Since the 1980s, many different antimicrobial substances in mollusks have been identified, described and characterized[39-42]. It has been demonstrated that small cysteine-rich peptides play a very important role in the defense system of molluscs[1,3,39,40,43-47]. Previously the antimicrobial activity of marine mollusks has been tested in many experiments. The potential and extent of the antimicrobial activity varied depending on the species of bacteria and the solvent used for extraction. Jayaseeli *et al*.[23] reported the antibacterial actions of 4 bivalves against a few pathogens, the most significant activity of the extracts being against *Bacillus subtilis*. In the present study, *P. perna* shows the highest activity against *B. subtilis* when compared to the other 2 extracts. Antibacterial activity of gastropods against *S. typhi* was reported by Rajaganapathi[48], which supports the results of the present study on the antibacterial activity of mussels extracts. Similarly in the present study mussel extracts show the highest activity against *Salmonella typhi* but the lowest activity against *S. paratyphi*. Annamalai *et al*.[26] reported that *Pernaviridis* extract shows the highest level of activity (4 mm) against *E. coli*. In contrast, the methanol extract of *P. viridis* in the present study created 14 and 13 mm of inhibition zones against *P. aeruginosa* and *E. coli* and showed less activity against acetone, hexane and butanol and this was coincided with the results of the present study. Compared to these studies, the methanolic extracts of *P. viridis*, *P. perna* and the parrot mussel show the higher degree of inhibition indicated that the substance involved in mussel species producing the antibacterial effect could be a high polar compound, because methanol is a highly polar solvent.

In the present investigation, the high antibacterial activities of *P. viridis*, *P. perna* and the parrot mussel tissues were observed. The difference in the antimicrobial activities found in mussel extracts of different species and solvents may depend on the extracting capacity of the solvents and the compounds extracted. Madhu *et al*.[49] reported that *P. viridis* has good antimicrobial potential. Also this study proves that compared to *P. viridis* the brown mussel *P. perna* has better antimicrobial potential and the parrot mussel shows intermediate responses.

In this study it was assumed that the antimicrobial compounds were basically proteins or peptides in nature, a combination of 'soft techniques' was selected for the preparation of crude extract using methanol, ethanol, acetone, hexane and butanol to ensure that the functionality or the biological activity of the analysis remained intact[50]. For this, methanol and ethanol were selected as suitable solvents as they have good extraction efficiency. Most of the low molecular weight proteins/peptides (stable at room temperature) are extracted using them as solvents and they have the added advantage of allowing rapid sample concentration through evaporation. The results of the antimicrobial assay in the present study indicate that these extracts show high antimicrobial activity against the tested pathogens; they further point to the fact that these procedures are capable of extracting the antimicrobial compound, with relatively higher activity, without degrading the nature of the compound. The crude extract has been reported to possess short peptides, free amino acids (conjugated with metals like Cu, Zn etc) and minerals. They were also reported to possess low fat and salt contents[51]. The antimicrobial activity of the extract might be either due to short peptides, amino acids conjugated with metal ions or both or it could also be due to the generation of artifacts during the extraction process. Gonzalez *et al*.[52] reported that the tissues of the oyster *Crassostrea gigas* contain antimicrobial peptide responsible for antibacterial activity.

The occurrence of bioactive substances, especially antimicrobial peptides in marine invertebrates and their high activity against pathogens point to a possible way towards new therapeutic agents. At the present juncture, the development of antimicrobial resistance by bacteria is one of the vexing problems worldwide. For this reason, there is a rising need to look for new options of therapy[40,41,45,53]. In the last decade alone, structures of over 5,000 marine natural products have been elucidated[54]. More than 100 pure compounds of known and new structural types have been isolated and characterized. These compounds belong to different structural types namely diterpenoids (37%), steroids/sterol glycosides (18%), sesquiterpenoids (17%), alkaloids, amino acids, fatty alcohol esters and glycolipids constituting the remaining portion[24]. Marketable antibiotics are greatly helpful to kill the bacterial pathogens involved in common infection. Mussel samples extracted by using different solvents in this study showed considerable antibacterial activity. The three mussel types were examined and each of them was found to have distinct ability to act against the pathogen. The product from the natural resource is fine for health and devoid of side effects. The study indicates that tissue extraction of *Perna* species would be a good

resource of antibacterial compounds and would substitute the existing inadequate and expensive antibiotics. However, additional investigations are required into the function of the extracts as the drug for human administration.

The method of FTIR analysis is a conformational study of antimicrobial peptides[55] and the crude antibacterial methanol extract used in this study reveals the presence of bioactive compounds signals at different ranges. The present study indicates the presence of the following in *P. viridis*, primary amide, Amide 2 and 3, aliphatic amines, C-methyl, an azide group. Further, *P. perna* has secondary amine, primary amide, C-methyl group, aldehyde and aliphatic amines. Similarly, the parrot mussel has the functional groups of primary amide, amide 2, amide 3, C-methyl, aldehyde, isocyanate and aliphatic amines. Most of the functional groups are similar between the 3 mussel species except azide, the azo group present in *P. viridis*, isocyanate group present in the parrot mussel. Earlier, in a similar study, the FT-IR analysis of crude venom of catfish *Arius maculatus* has inferred the presence of aromatic primary amine, aromatic tertiary amine CN 115 stretch, primary amine CN stretch. It is also reported to contain alcohol group at 576 cm-1. Further, the sample contains aldehyde group, alkenyl C=C stretch, methyl C-H bond, methylene groups C-H band, respectively[56].

In the present investigation *P. viridis*, *P. perna* and the parrot mussel have second derivative spectrum of peptides (amide 1 and 2), aldehydes and aliphatic amines. Madhu *et al.*[49] reported amide (1-5) compound as a functional group in *P. viridis* responsible for antibacterial activity and this coincides with our result. Azo group compounds are responsible for biological activity[57]. This azo group is responsible for the colour of the substrates and in the present study azo group was only found in *P. viridis*. Tyrian purple is a famous and expensive animal-based dye obtained from shellfish nurez and madder root produces a brilliant red colour. Both these dyes have azo compound[58].

The samples of the mussels *P. viridis*, *P. perna* and the parrot mussel showed broad amide 1 (A1) bands at 1645, 1638 and 1639^{-1} and this property is typical of proteins with high a-helical content. The high ratio of A2-1 bands in the mussel samples can be attributed to the high content of α-helix. Gorinstein *et al.*[59] observed the amide bands in mussel *Mytilus galloprovincialis* by FTIR. The band of high-frequency components in the amide I band can be assigned to turns and elements of h-sheet[60]. The whole contribution to the amide I and II bands is estimated at around 20% of the total absorbance. Gorinstein *et al.*[59] reported that the detection of band at 1632 cm^{-1} was not shown in dirty sample. In this study also the similar range of peak was observed in the sample indicated that the collection site of the sample (Kadiyapattinam coast) was free from pollution. Isocyanate was found only in the parrot mussel but it was not found in *P. perna* and *P. viridis* and this functional group may differentiate the parrot mussel from the other species. The isocyanate is one of the compounds of diterpenoid and the biosynthesis of diterpenoid has been extensively studied in plants, fungi and bacteria. They have been now recognized for the discovery of new natural products[61]. Wright *et al.*[54] and Konig *et al.*[62] isolated diterpenoids containing isothiocyanate group in marine sponge *Cymbastela hooperi*. The diterpenoid isocyanate showed higher activity against bacteria, especially against gram-negative bacteria. The present study revealed that the experimental mussel samples have the promising new lead compound for antimicrobial drugs.

CONCLUSION

Many diseases are initially controlled exclusively by the use of antimicrobial drugs. The massive use of antimicrobials for disease control and growth promotion in animals increases the selective pressure exerted on the natural emergence of bacterial resistance. So, there is an urgent need for the discovery of new and novel antimicrobial drugs to effectively combat not only the drug resistance but also the new disease producers. Hence, the search for active drugs from alternative sources including marine environment obviously becomes imperative. All the 3 mussels in this study showed maximum activity against tested pathogens and the differences were not remarkable between the mussel species. This finding is very significant and may pave the way for the discovery of new potent drugs against the dangerous pathogens. Based on the count of their broad-spectrum antibacterial activity and taking into account the previous available literature, we can safely assert that *P. viridis*, *P. perna* and the parrot mussel may be expected to be potential producers of new antibiotics.

SIGNIFICANCE STATEMENT

This study discovered the bioactive properties of Mussels species that can be beneficial for discovery of new potent drugs against the dangerous pathogens and this study will help the researchers to uncover the critical areas of antimicrobial drugs that many researchers were not able to explore. Thus a new theory on pharmaceutical agents may be arrived at.

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