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Research Article

Exploration for Antimicrobial Susceptibility of Proteins from Edible Marine Source *Himantura gerrardi*

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ABSTRACT

Marine organisms represent a valuable source of new compounds. The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of new active substances in the field of the development of bioactive products. Protein rich consumable marine fish named Stingray fish (Thirukkai meen) was collected from the seashore of Kanyakumari. Tissue and skin extracts were separated with the intention to identify antimicrobial proteins for the study. Tissue and skin were extracted by acetic acid, and it was partially purified using Ammonium sulphate precipitation method. Antimicrobial activity was done, and a maximum zone of inhibition was observed in *Staphylococcus aureus* with tissue extract and a maximum zone of inhibition was found in *Klebsiella pneumoniae* with skin extract by agar well diffusion method. The crude protein was quantified with 2 mg/ml for tissue extract and 1.26 mg/ml for skin extract. The FTIR analysis also revealed the presence of secondary amines in the crude extracts of tissue and skin. Time kill kinetics showed the results that the live bacterial cells were killed at the minimal concentration of the drug. Tissue extract showed a molecular weight ranging from 150 K.Da to10 K.Da and skin extract ranges below 25 K.Da by SDS-PAGE analysis. Agar overlay assay made possible to estimate the amount of antimicrobial activities of the proteins in a semi-quantitative manner and to identify such significant antibacterial activities in tissue extract. The molecular weight of tissue fraction from the obtained mass spectrum was about (app 10 K.Da), as determined by MALDI-TOF MS analysis. The antimicrobial proteins/peptides identified from these edible sources were found to be promising antibiotics that may replace the existing synthetic antibiotics with side effects.

Keywords: Stingray fish, Antimicrobial peptides, Antimicrobial activity

INTRODUCTION

Edible marine sources are the important part of the human diet. There are 32 phyla that are present in the sea (approximately) and along with these, the Marine organisms represent a majorly unexplored new area, having highest chances for the identification of compounds with higher potency and novel biological actions. This has been shown the approach of isolating novel chemical structures and compounds from marine natural products (MNPs) whereby MNPs has yielded a significant number of drug candidates with successful antimicrobial (both antibacterial and antifungal) properties with novel mechanisms of action. A marine natural product has medicinal benefits and so many marine natural products have been exposed, and some drugs are under clinical trials. It also has other properties like anticoagulant, anticancer, immune-suppressants, etc.[1]. The ubiquitous amount of antimicrobial peptides which are present in marine environment attests to their overall importance in building the defence mechanism in most of the organisms. Antimicrobial peptides are precise part of the humoral natural defence of invertebrates against infections and hence they are also been termed as “natural antibiotics”. Antimicrobial peptides are present in marine sources are small, amphipathic and cationic molecules[2]. Hence it has the ability to reduce the effect the pathogenic microorganism. They have innate mechanism of action[3]. There is a vital interest in discovering new antimicrobial compounds with fewer environmental and toxicological risks and no resistance developed by the pathogens. In marine invertebrates so far approximately 7000 marine natural products have been reported, 33% from sponges, 18% from coelenterates, and 24% from representatives of other invertebrate phyla such as ascidians, opistho branch mollusks, echinoderms and bryozoans[4]. So in the current work is to identify the antimicrobial properties of sting ray fish against human pathogenic strains and to confirm the presence of protein in the sting ray fish.

MATERIALS AND METHODS

Collection of edible marine source

Edible marine source was collected from sea shore of Kanyakumari. The marine source selected for study was sting ray fish and it was authenticated by Dr. A.Shanmugam, Professor, CAS in Marine Biology, Annamalai University, Parangipettai. The samples were identified as *Himantura gerrardi* of the family Dasyatidae

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and their parts: Tissue and skin were separated for the study.

Extraction of crude protein

For extraction of protein from edible marine fish *Dasyatidae sp.*, the tissue and skin was freshly taken and homogenized with acetic acid and the homogenate was centrifuged at 12000 x g for 20 min (to remove cellular debris) in a cooling centrifuge. The pellet was discarded and the supernatant was used in subsequent steps. The protein in supernatant was concentrated by ammonium sulfate precipitation method. The precipitate was centrifuged at 12000 x g for 20 min and dialysed extensively against deionized water and stored at -20˚C until evaluation[5].

Antimicrobial activity

The antimicrobial activity was carried out using Agar well diffusion method against 10 bacterial strains viz., *Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Shigella flexneri, Salmonella typhi, Bacillus subtilis, and Vibrio cholerae* and 4 fungal strains viz., *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Cryptococcus neoformans*. Nutrient Agar and Sabouraud dextrose agar plates was swabbed (sterile cotton swabs) with 8 hour old broth culture of respective bacteria and fungi. Wells was made in each of these plates using sterile cork borer. The crude protein extracts of tissue and skin at different concentrations (25 µl, 50 µl,75 µl and 100 µl) was added into the wells. The plates were incubated at 37°C for 24 h for bacterial pathogens. Triplicates were maintained and the experiment was repeated thrice. The diameter of the inhibition zone (mm) was measured and the mean and standard deviation was also calculated[6].

Determination of MIC

Lowest concentration of active principle compound preventing microbial growth was considered to be the MIC. Minimal inhibitory concentrations of crude proteins were determined against both Gram negative and Gram positive bacteria by microplate assay method, in sterilized 96-well plates (NUNC, Roskilde, Denmark). The final volume of 300 µl composed of 50 µl of bacterial culture, different concentrations of Nutrient Broth medium (50ul,75ul,100ul,125ul,150ul,175ul,200uland 225ul) and different concentrations of protein extracts (25ul,50ul,75ul,100ul,125ul,150ul,175ul and 200ul). MIC was recorded in Microplate reader (Epoch) at wavelength of 250 nm after 24 hrs of incubation at 37° C[6].

Determination of MBC

MBC was determined by transferring and spreading the samples from MIC plates. The least concentration of protein extract showing no observable growth on nutrient agar plates after incubated at 37° C for 24 hrs.

Time kill kinetics assay

Time-kill kinetics assay was done by microplate method. Microplate method was performed in sterile, flat-bottomed 96-well microplates. The crude extract was diluted with different concentrations (50µl, 75 µl, 100 µl, 125 µl, 150 µl, 175 µl, 200 µl and 225 µl) of Nutrient Broth and 50ul of culture to obtain two times the final concentrations. Bacterial suspension (100 µL) was added to each well, resulting in the desired final drug concentration and inoculum size. Addition of an equal volume of sterilized nutrient broth was used as growth controls, respectively. The microplates were incubated for 24 h at 35 °C and reading was taken for every 3 hr with the microplate reader (Epoch). Data were obtained from three independent assays performed in duplicate. The absorbance was noted in all the wells and the OD was calculated as per the formula below[7].

$$\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}}$$

Determination of protein`s by Lowry's method

The protein content of precipitated crude protein samples was estimated by Lowry's method using Bovine Serum Albumin as standard[8].

SDS Page analysis

Samples was separated by SDS-PAGE it was treated as follows: 10 µl of loading buffer (containing β-mercaptoethanol, SDS, glycerol, Tris-Cl and bromophenol blue) was added to 30µl of crude protein extract and the mixture was heated for 10 min at 100˚C. The samples were loaded onto a precast, gradient, 12% polyacrylamide gel using MOPS running buffer containing 4µl of reducing agent (1×). The gel was fixed in a 40% methanol, 10% acetic acid solution and stained with colloidal Coomassie brilliant blue stained gels was visualized for the separated bands[9].

Agar-overlay assay

SDS containing gels was subjected to agar overlay assay. each gel slice was taken and it was fixed for 2 hours by using a fixation solution containing 20% isopropanol, 10% acetic acid in sterile Distilled water and then it was washed by about 300 ml of sterile distilled water for additional 3h at room temperature (6 times, 50 ml each). Bacterial strains were grown overnight on Nutrient agar plates at 35ºC. Each washed gel was placed on a sterile Petri dish which contains 25 ml of nutrient agar medium and the test microorganism was swabbed over the gel. Incubation was done at 35ºC for 20 h.

A SDS gel was also run just with the sample buffer and Distilled water as negative control for investigation of the probable effects of sample buffer components or trace amounts of remaining SDS in antimicrobial assays for each tested microorganism. This gel also was fixed and washed according to the above described protocol[10].

FTIR-Analysis

The crude protein extract was subjected to FTIR characterisation under suitable conditions. The Functional groups of proteins was identified in a different wavelength. 100mg dried of potassium bromide was compressed to prepare a salt disc (10 mm disc) and sample of 1 drop was kept over the disc for spectrometer reading .The infrared absorption bands identified molecular components and structures.

MALDI TOF analysis

The MALDI-TOF mass spectra were used for the analysis of peptide mass fingerprinting and MS/MS ion search. The

Table: 1 Antibacterial activity of tissue extracts

S. No	Bacterial pathogens	50µl	75µl	100µl	PC (Chloramphenicol 10 mg/ml)
1	*Streptococcus pyogenes*	6.33±0.57	9±0.58	10.33±0.99	8±0.49
2	*Staphylococcus aureus*	6.55±0.11	10.41±0.99	16.83±0.76	14.83±0.28
3	*Klebsiella pneumoniae*	6.4±0.52	8.5±0.5	12.16±0.12	9.5±0.15
4	*Escherichia coli*	6.06±0.81	7.41±0.79	7.93±0.77	8.9±0.71
5	*Pseudomonas aeruginosa*	8.71±0.54	10.95±0.72	13.27±0.58	13.25±0.52
6	*Proteus vulgaris*	NA	NA	NA	6.9±0.89
7	*Shigella flexneri*	7.25±0.75	9.6±0.70	13.35±0.56	11.12±0.52
8	*Salmonella typhi*	8±0.32	9.06±0.37	11.08±0.57	13.33±0.55
9	*Bacillus subtilis*	7.55±0.63	7.10±0.43	12.03±0.56	10.63±0.65
10	*Vibrio chlorae*	6.35±0.59	9.96±0.48	11.570.63	14.22±0.54

NA – no activity

Table: 2 Antibacterial activity of skin extract

S. No	Bacterial pathogens	50µl	75µl	100µl	PC (Chloramphenicol 10 mg/ml)
1	*Streptococcus pyogenes*	7.26±0.65	9.9±0.46	11.88±0.51	10.01±0.42
2	*Staphylococcus aureus*	7.3±0.44	8.9±0.42	10.13±0.60	7.8±0.51
3	*Klebsiella pneumoniae*	6.9±0.62	9.06±0.50	12±0.34	12.98±0.23
4	*Escherichia coli*	7.9±0.25	9.96±0.40	11.02±0.29	13.18±0.40
5	*Pseudomonas aeruginosa*	8.1±0.49	9.9±0.37	9.9±0.61	13±0.55
6	*Proteus vulgaris*	NA	NA	NA	7.1±0.37
7	*Shigella flexneri*	6.6±0.41	10.01±0.34	10.88±0.42	12.93±0.35
8	*Salmonella typhi*	0.0	8.8±0.45	10.05±0.28	12.23±0.49
9	*Bacillus subtilis*	7.08±0.34	9.03±0.28	10.05±0.48	11.92±0.43
10	*Vibrio chlorae*	6.6±0.35	10.68±0.45	11.97±0.41	13.82±0.28

NA – no activity

trypsin digested purified protein solution was used for the MALDI-TOF MS analysis, recorded on an AB SCIEX Voyager DE Pro MALDI-TOF (Applied Biosystems, USA) time-of-flight spectrometer, with a pulsed nitrogen laser (337 nm, 3-ns pulse width). The spectrum was recorded in the linear, positive high-mass mode. The pure lyophilized peptide fraction of 1µL was diluted with 2 µL of alpha-cyano-4-hydroxycinnamic acid and formed as a peptide matrix and transferred to a stainless steel target and dried under gentle vacuum. The steel plate was then washed with 1 µl of 0.1 % trifluoroacetic acid[11].

RESULTS AND DISCUSSION

Antibacterial activity of tissue extract

Results were expressed with mean and standard deviations by using Microsoft excel and it has shown in table 1. The maximum zone of inhibition 16.83±0.76 was observed for *S. aureus* at the concentration of 100 µl of crude tissue extract obtained by acetic acid and minimum was observed in 6.06±0.81for *E. coli* at the concentration of 50 µl and no activity was observed for *P. vulgaris* .The crude acetic acid extracts of tissue samples showed high antimicrobial activity against pathogenic isolates of *S.aureus, P. aeruginosa, V.chlorae* and *K. pneumonia.* Moderate activity also obtained for *Bacillus subtilis, E. coli, S. typhi, Shigella flexneri* and *Streptococuus sp*. No Activity was observed in *P. vulgaris.* The maximum zone of inhibition was observed in *Staphylococcus aureus* as 17mm for 100 µl of extract and the minimum zone of inhibition was observed in *E. coli* as 6mm for 100 µl of extract.

Antibacterial activity of skin extract

Results for the activity of skin extract against human pathogens at various concentrations and zone of inhibition (mm) with Mean and SD were interpreted in Table 2. Results were expressed with mean and standard deviation by using Microsoft excel. The maximum zone of inhibition 12±0.34 was observed for *K. pneumonia* at the concentration of 100 µl of crude skin extract obtained by acetic acid and minimum zone of inhibition was observed as 6.6±0.35 for *V.chlorae* at the concentration of 50 µl and no activity was observed for *P. vulgaris* .The crude acetic acid extracts of skin samples showed high antimicrobial activity against pathogenic isolates of *S.aureus, P. aeruginosa, Streptococuus sp* and *K. pneumonia.* Moderate activity also obtained for *Bacillus subtilis, Shigella flexneri S. typhi* and *E.coli*. No Activity were observed in *P. vulgaris.* The maximum zone of inhibition was observed in *K. pneumonia* as 12mm for 100 µl of extract and the minimum zone of inhibition was observed in *V.chlorae* as 6.6mm for 50 µl of extract.

Kaliyamoorthy *et al* reported that Sting ray fish *Himantura imbricata* was extracted using solvents of acetone, chloroform, ethanol and methanol of sting ray crude spine were tested against 10 human pathogens. Among that tested samples, maximum zone of inhibition of 14 mm was recorded against S.aureus and a very loweset zone of inhibition not in the considerable range was reported against E. coli by chloroform extracts.

The male and female haemolymph of the crab *Charybdis lucifera* collected from the Vellar estuarine environment

Table 3: Antifungal activity of crude tissue extract

S. No	Fungal pathogens	PC (Griseofulvin 10mg/ml)	50µL	75µL	100µL
1	*Cryptococcus neoformans*	10.93±0.62	NA	6.8±0.43	8.1±0.59
2	*Aspergillus niger*	11.97±0.41	7.26±0.44	8.85±0.39	10.12±0.33
3	*Aspergillus flavus*	10.97±0.25	NA	7.06±0.21	8.88±0.26
4	*Fusarium oxysporum*	10.90±0.28	NA	6.35±0.30	8.72±0.12

NA – no activity

Table 4: Antifungal activity of crude skin extract

S. No	Fungal pathogens	PC (Griseofulvin 10mg/ml)	50µL	75µL	100µL
1	*Cryptococcus neoformans*	11.97±0.55	NA	7.7±0.38	11.88±0.51
2	*Aspergillus niger*	11.97±0.27	NA	8.11±0.29	11.95±0.66
3	*Aspergillus flavus*	12.90±0.28	NA	7.85±0.35	10.47±0.47
4	*Fusarium oxysporum*	8.18±0.50	NA	6.5±0.42	9.10±0.40

NA-no activity

was reported with the antibacterial activity with the highest zone of inhibition from male crab (11 mm) against *Escherichia coli* and female crab against *Pseudomonas aeruginosa*. The lowest zone of inhibition was recorded in female crab (7mm) against *Vibrio sp*. But the fungal activity 10 different strains was *Fusarium moniliforme* showing activity (10 mm) rest of them are not showing any activity[13].

Antifungal activity of crude tissue extract

The radius of inhibition zone for crude tissue extract has shown in table 3. Results were expressed with mean and standard deviation by using Microsoft excel. The maximum zone of inhibition 10.12±0.33 was observed for *Aspergillus niger* at the concentration of 100 µl of crude tissue extract obtained by acetic acid and minimum was observed in 6.35±0.30 for *Fusarium oxysporum* at the concentration of 75 µl. The crude acetic acid extracts of tissue samples showed high antifungal activity against pathogenic isolates of *Aspergillus niger* and *Aspergillus flavus.*

Moderate activity also obtained for *Cryptococcus neoformans* and *Fusarium oxysporum.* The maximum zone of inhibition was observed in *Aspergillus niger* as 10mm for 100µl of extract and the minimum zone of inhibition was observed in *Fusarium oxysporum* as 6.3mm for 75µl of extract.

Antifungal activity of skin

The radius of inhibition zone for crude skin extract has shown in table 4. Results were expressed with mean and standard deviation by using Microsoft excel. The maximum zone of inhibition was observed in11.95±0.66 for *Aspergillus niger* at the concentration of 100 µl of crude skin extract obtained by acetic acid and minimum was observed in 6.5±0.40 for *Fusarium oxysporum* at the concentration of 75 µl. The crude acetic acid extracts of skin samples showed high antifungal activity against pathogenic isolates of *Aspergillus Flavus and Cryptococcus neoformans.* Moderate activity also obtained for *Aspergillus Niger* and *Fusarium oxysporum.* The maximum zone of inhibition was observed in *Aspergillus Flavus and Cryptococcus neoformans* as 7.7mm for 100µl of extract and the minimum zone of inhibition was observed in *Fusarium oxysporum* as 6.5mm for 75µl of extract. The cat fish mucus has shown antifungal activity against ten fungal pathogens like *Pencillium sp. Trichophyton mentagrophytes, T. rubrum,* pathogenic fungal strains. The maximum antifungal was observed against *C. albicans (12mm)* and *alternaria, A. flavus, A. niger* minimum was against *Mucor sp.* (6.5mm) in the mucus of cat fish[14].

Table 5: Minimum inhibitory concentration

S.No	Extracts of Protein	MIC(mg/ml)
1	Tissue	0.83
2	Skin	0.09

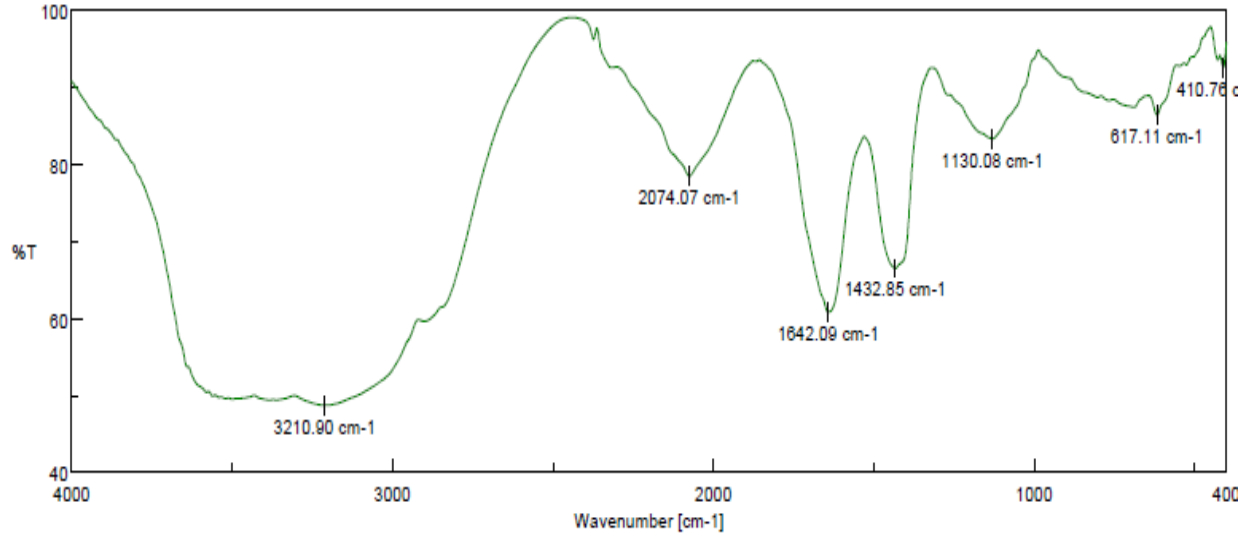
Minimum inhibitory concentration

The MIC was determined by micro dilution assay for the two protein extracts at different concentrations ranging from 15ul to 200ul revealed the results that all the tested pathogenic bacteria were inhibited by the crude extract at 25ul the concentration of protein in tissue extract was 0.83 mg in 25ul and skin extract was 0.09 mg in 25ul as given in table 5.The growth of all the bacterial pathogens were inhibited at these least concentrations of proteins which helps us to better understand the potency of crude protein extract with good antibacterial nature. The antibacterial activities of stingray fish may be due to the presence of antibacterial glycoproteins and able to kill the bacteria by forming pores in the target membrane.

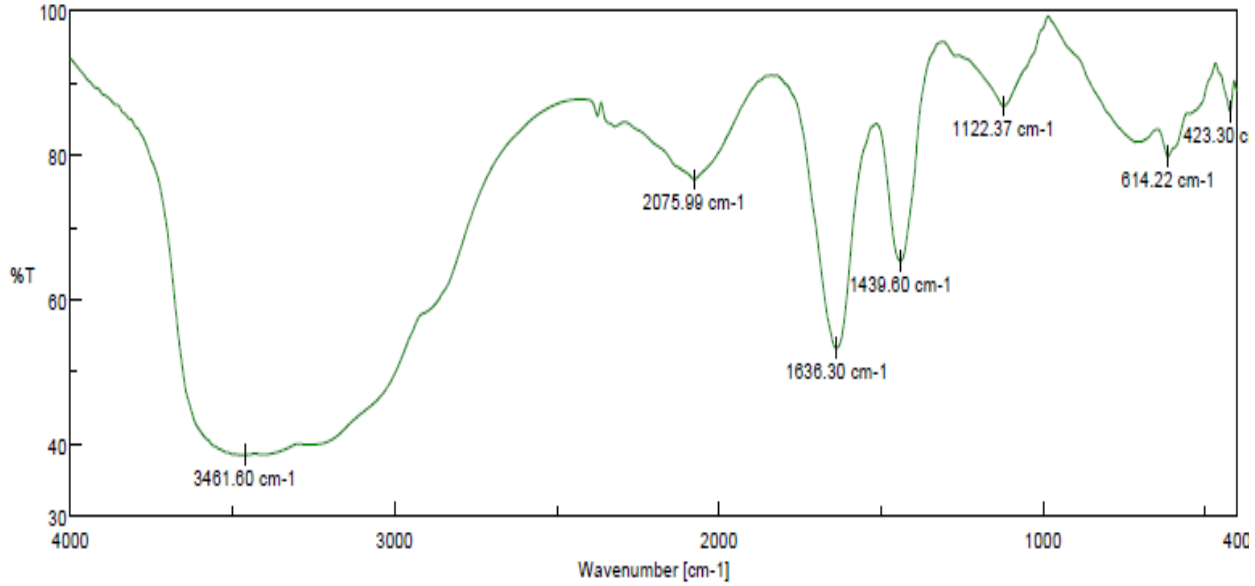
MIC values of antimicrobials observed in the study were reported by Tracy *et al*. Antibacterial susceptibility testing using crab shell extract showed that only *Klebsiella pneumoniae* were sensitive while *P. mirabilis* were resistant. The average minimum inhibitory concentration [MIC] of *Klebsiella pneumoniae* was determined to be 10.42 µg/ml.

Minimum bactericidal concentration

The MIC samples subjected to MBC to determine the bactericidal concentration showed the cidal effect of the extracts from the least concentration of the extract at 25 µl. The lowest concentration of extract that showed no bacterial growth on agar plate represents the MBC value of both the extracts. The minimum bactericidal



Graph 3: FTIR Analysis for tissue extract



Graph 4: FTIR Analysis for skin Extract

concentration was showed as 0.10 mg/ml for tissue extract and 0.09 mg/ml for skin extract.

Protein estimation by Lowry`s method

The Acetic acid tissue and skin extracts of sting ray fish were estimated for their protein concentration by Lowry's method. The concentration of crude protein was calculated from the standard graph of BSA. The optical density of the extracts at various concentrations revealed the amount of protein in the extract. The crude protein was quantified with 2 mg/ml of protein for tissue extract and 1.26 mg/ml of protein for skin extract.

FTIR characterisation

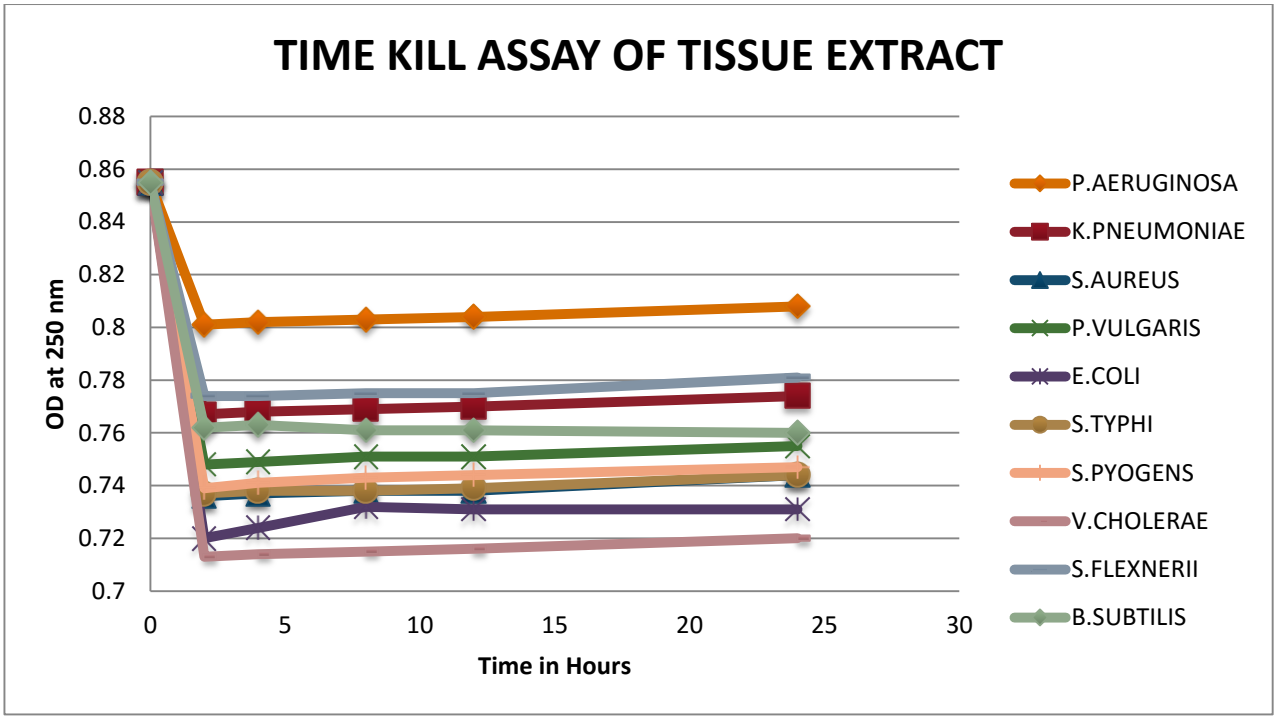
The FTIR analysis also revealed the presence of secondary amines in the crude extracts of tissue and skin compared to the solvent systems used for extraction in the below graphs.

FTIR characterization of crude protein showed the presence of functional groups of protein like NH, COOH, -CO-, C=C and other R groups at appropriate wave length and stretching. Tissue and skin extract was analysed by FTIR in graph 3 and 4.It has –C = C–H: C–H alkynes (2047 cm^{-1}), –C = C– alkenes (1642 cm^{-1}), COOH Carboxylic acids (1432 cm^{-1}), –CH2X alkyl halides (1130 cm^{-1}), –C = C–H: C–H alkynes (617cm^{-1}).

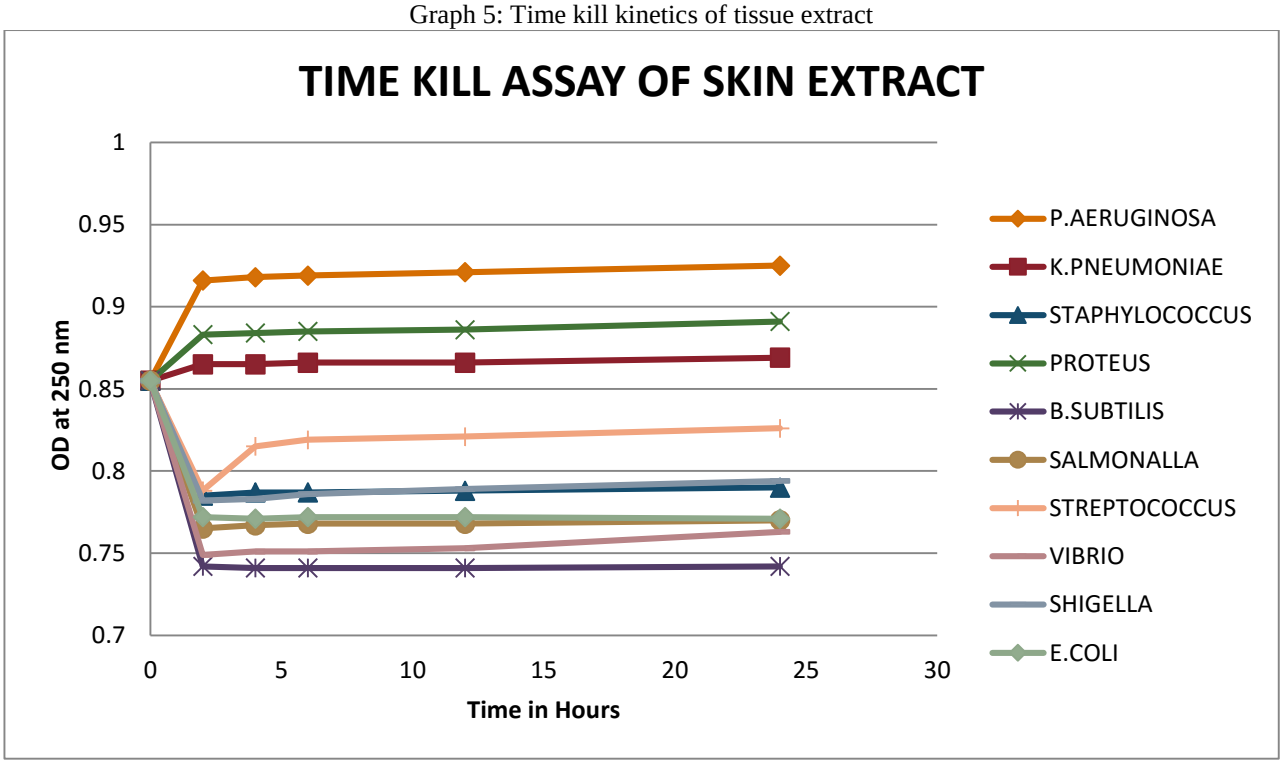
Time kill kinetics of tissue extract

The kinetics of tissue protein extract measured for duration of 24 hrs at six different time intervals were observed with considerable decreasing OD values which revealed the results that the concentration of live bacterial cells were decreased from initial concentration. The kinetics of all the pathogens showed decreasing values compared to the tissue extracts. The value of *E.coli* was decreased after slight increase of the value.

Farrukh *et al* reported that the findings of the *in vitro* time-kill assay of the most active compound, verongiaquinol, against *S. aureus* indicated its inhibitory effect at a level



Graph 5: Time kill kinetics of tissue extract



Graph 6: Time kill kinetic assay of skin extract

lower than the MIC values (i.e., 2 and 4 µg/mL concentration). At the MIC (8 µg/mL), bacterial cells were completely killed within 18 hours of incubation. It is interesting to note that verongiaquinol exhibited activity within 2 hour of exposure.

Time kill kinetics of skin

The kinetics of crab protein extract measured for duration of 24 hrs at six different time intervals were observed with considerable decreasing OD values which revealed the results that the concentration of live bacterial cells were decreased from initial concentration. The kinetics of four pathogens *Proteus sp, Klebsiella sp, Pseudomonas sp* and *Streptococcus sp* were increased and remains constant after the following hours. All the other pathogens showed decreasing values and remains constant after one hour. The value of *E.coli* was decreased after slight increase of the value.

Wang *et al* reported in the kinetic study that a highly sensitive strain of S. aureus was used to evaluate bactericidal activity of the synthetic PC-hepc. The

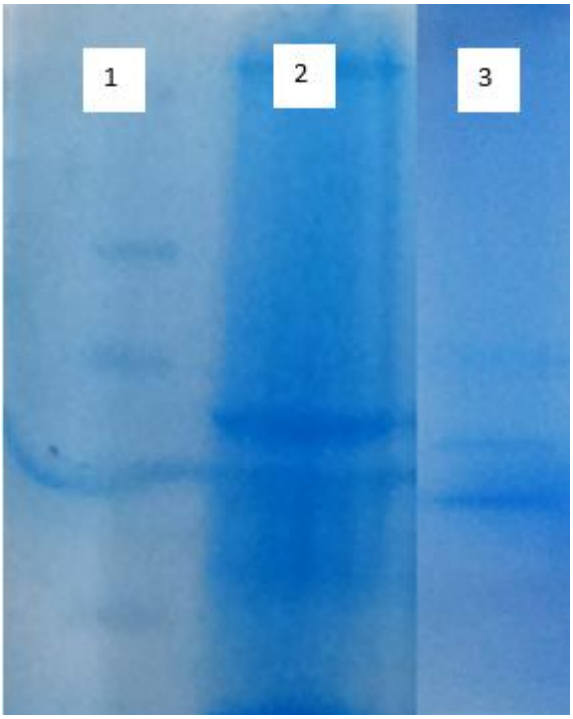


Figure 1: SDS page analysis
Lane 1: Protein Marker, lane 2: tissue extract, lane 3: skin extract

bactericidal activity of this peptide was assessed by plating cultures and the number of CFUs was countered overnight incubation at 37 8C. When S. aureus was incubated with the synthetic PC-hepc at a concentration 1–2 times the MBC value (12– 24 mM), approximately 90% bacteria were killed at 9 min and nearly all the bacteria were killed around 30 min.

SDS page analysis

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis is an excellent tool to identify and monitor proteins during purification and to access the homogeneity. The crude protein extracts of tissue and skin were analysed for the molecular weight of proteins in the extracts. The standard was loaded in Lane 1 followed by tissue extract in Lane 2, skin extract in Lane 3 (Fig: 1). All the other lanes were left unloaded. The electrophoresed gel was documented in Gel Doc (Bio Rad). The intense bands were marked with pink lines by the documentation system. The Biorad precision plus unstained protein marker was used as the standard. The molecular weight of standard ranges from10 K.Da to 250 K.Da. The tissue extract showed the presence of 6 bands with molecular weight ranging from 150 K.Da to10 K.Da. The skin extract in Lane 3 was observed with the 3 bands and most of them have molecular weight below 25 K.Da.

In Effect of alkaline pH on bioactive molecules of epidermal mucus from *Labeo rohita* (Rahu) Najiya *et al* reported that Prominent peptide bands ranged between 13 KDa to 100 KDa were observed in anaesthesia treated mucus as compared to alkali treatment that denatured the most of the bands.

Agar overlay assay

In agar-overlay assays, there were clear and noticeable zones of inhibition in the tested gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Klebsiella pneumoniae* The tissue extract showed the powerful antibacterial activities against tested gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Klebsiella pneumoniae.* But in skin extract there was zone of inhibition in gram positive bacteria *Staphylococcus aureus* and no zone was found in gram negative bacteria *Klebsiella pneumoniae* when agar overlay assay performed. This approach made it possible to estimate the amount of antimicrobial activities of the proteins in a semi-quantitative manner and to detect such great antibacterial activities in tissue extract.

MALDI TOF analysis

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF/TOF MS) analysis of tissue fraction was performed with different ion signals in the mass range of 400–5200 Da.

The molecular weight of tissue fraction from the obtained mass spectrum was about 9943.67 Da (app 10 KDa), as determined by MALDI TOF MS analysis.

CONCLUSION

Marine source sting ray fish chosen for study produces good activity in the trial. The tissue and skin of stingray fish selected for the study. Tissue and skin was extracted by acetic acid and partial purification of proteins was done by ammonium sulphate precipitation method. Their antimicrobial activities was carried out against Gram positive, Gram negative and fungal pathogens by agar well diffusion method and were observed to have good antimicrobial potency against all the tested pathogens. The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were also found to be effective at very low concentrations of all the extracts. The crude proteins were estimated with high protein concentration. The SDS PAGE and FTIR analysis confirmed the presence of proteins in the crude extracts. Agar overlay approach made it possible to estimate the amount of antimicrobial activities of the proteins in a semi-quantitative manner and confirm great antibacterial activities in the extract. The molecular weight of tissue fraction from the obtained mass spectrum was about (app 10 K.Da), as determined by MALDI TOF MS analysis. The antimicrobial proteins/peptides identified from these edible sources were found to be promising antibiotics which may replace the existing synthetic antibiotics with side effects.

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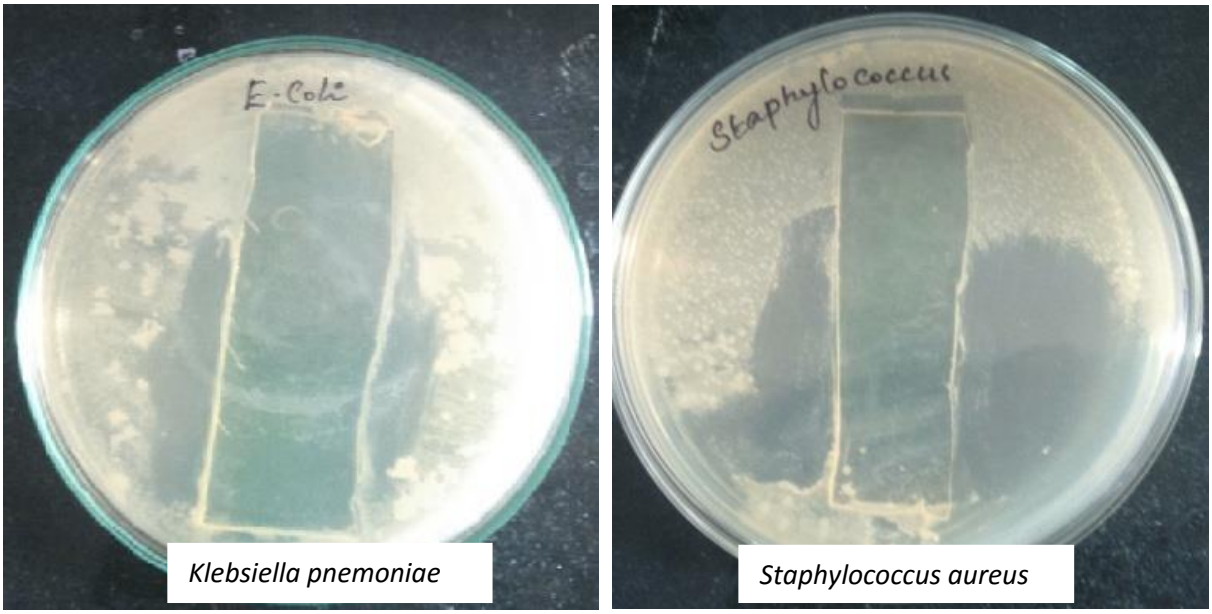


Figure 2: Plates showing the activities of tissue extract in agar overlay assay

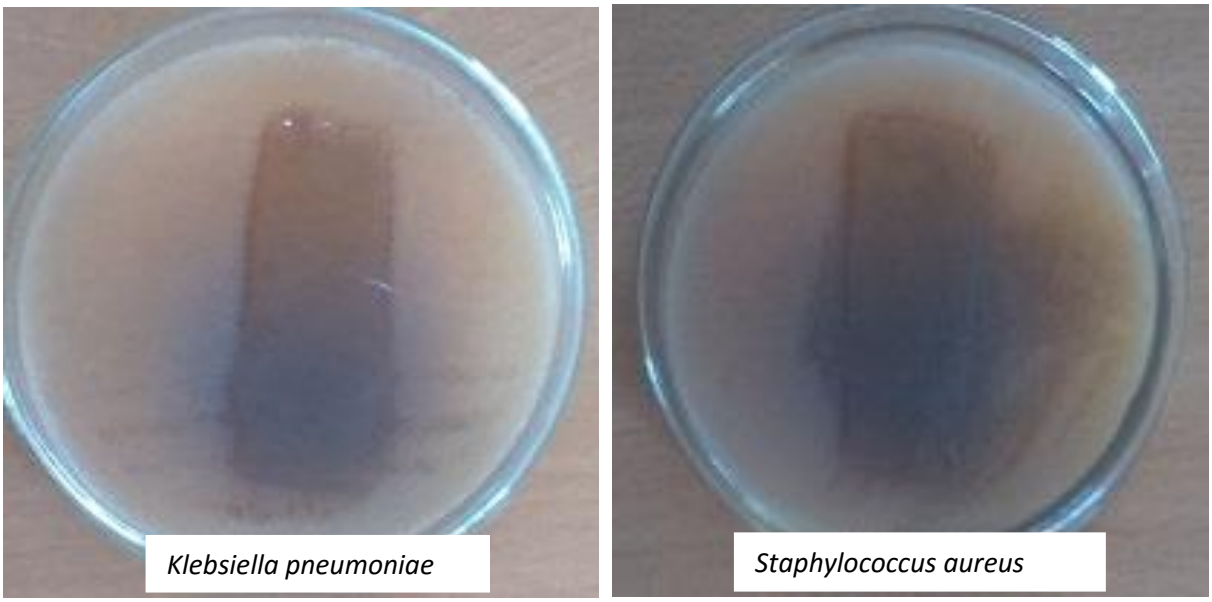
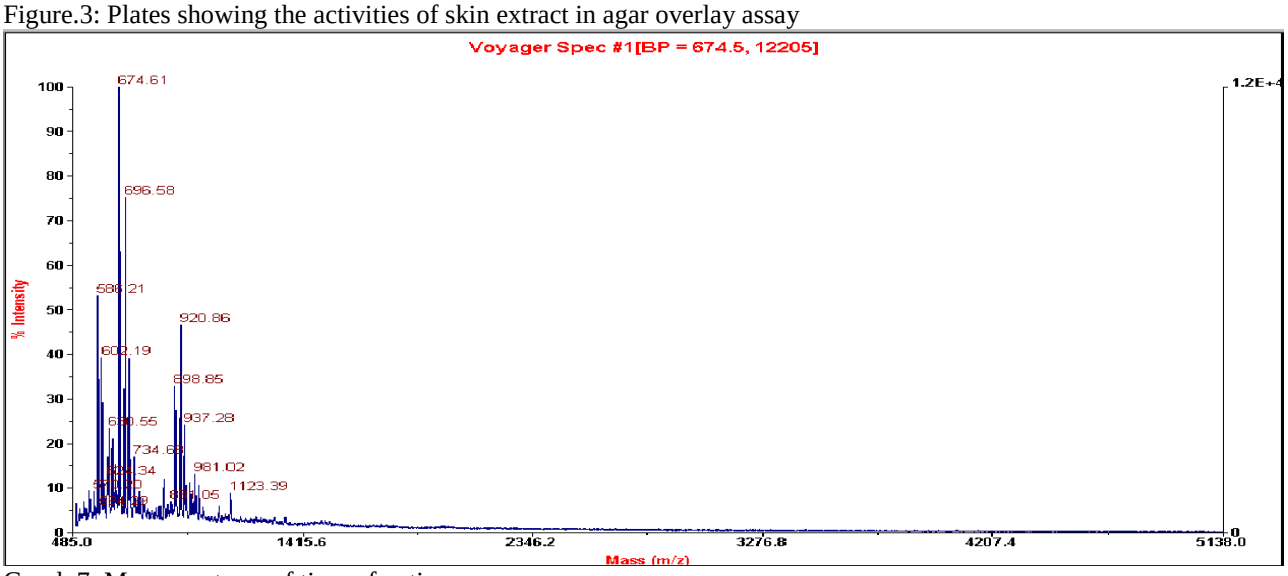


Figure.3: Plates showing the activities of skin extract in agar overlay assay



Graph 7: Mass spectrum of tissue fraction

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