Seaweed Res. Utiln., 38(2) : 65 - 67, 2016

Antibacterial activity of seaweeds collected from Olaikuda, Rameswaram, southeast coast of India

SUPARNA ROY* AND P. ANANTHARAMAN
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University
Parangepettai - 608 502, Tamil Nadu, India
**Email : suparna09roy@gmail.com*

ABSTRACT

The results obtained on the microbial activity of *Chaetomorpha antennina* and*Amphiroa anceps* collected from Olaikuda coast, Rameswaram are presented and discussed in this paper.

Introduction

Marine macroalgae commonly known as seaweeds have been recognized as potential sources of the antibiotic substances. A wide range of bio-active compounds were derived from macro algae. Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as anti-microbial, antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-hypertensive etc. They are renewable natural resources, abundantly growing along the coastal area of the world including islands and reefs. The seaweeds occupy a special sit as a source of biomedical compounds and potential source of antibiotics. Harder (1917) was the pioneer to observe the antimicrobial potentials of seaweeds. Many algal species have been shown to have bactericidal or bacteriostatic substances.

Materials and Methods

Two seaweeds viz., Chaetomorpha antennina (Chlorophyta) Fig. 1. and Amphiroa anceps (Rhodophyta) Fig. 2. were collected from Olaikuda coast near Rameswaram (Fig. 3). They were shade dried and used for anti-bacterial activities to the human pathogens such as Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli obtained from the Dept. of Microbiology, Raja Muthiah Medical College, Annamalai University. The accurately weighted powder seaweeds of 250mg were cold extracted for 2 hours vortexing in every alternative day in 20ml distilled water,

Fig. 1. *Chaetomorpha antennina*

Fig. 2. *Amphiroa fragilissima*

Received : Sep. 2016; Revised : Oct. 2016; Accepted : Nov. 2016

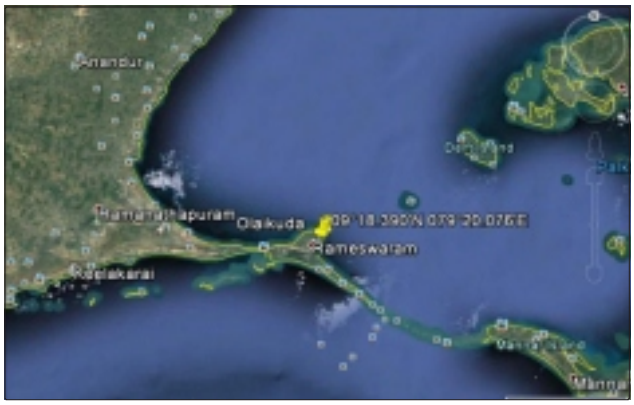

Fig. 3. *Map showing the collection locality*

chloroform, acetone, ethanol, ethyl acetate and Dimethyl sulfoxide (DMSO) for one week. The extracts were filtered and filtrates were collected for anti-microbial assay. The zones of inhibitions were measured using agar diffusion method with positive control as ciprofloxacin, amoxicillin and ampicilin. Both seaweeds species showed antibacterial activity depending on the solvent used. The DMSO extract of both seaweeds showed antibacterial activities to all three pathogens followed by water and acetone. The seaweed extracts were analysed between 300nm -700nm in UV-Vis spectrophotometer for preliminary assays for compositional aspects.

Dried seaweeds powder accurately weighted of 250 mg were dissolved in 20 ml of water, methanol, acetone, ethanol and diethyl ether and concentrated Dimethyl Sulphoxide (DMSO), the mixed solutions were kept for 7 days for cold extraction and vortex every alternative day for 2 hours in a screw cap brown bottle and the bottle kept in dry dark cool place in room temperature. After 7 days of cold extraction, the solutions were filtered with Whatman (589/2) filter paper and the crude extracts were used for assay anti-microbial activity to the selected human pathogenic bacteria. Antibacterial activity was evaluated by agar diffusion method (Bauer *et al.* 1966). In sterile agar plates, pathogens 200 µl broth culture were added to it and allowed to grow for 24 hours. Then, each crude extracts of seaweeds of 20µl were poured into the sterile discs and placed on the pathogenic agar plates. Each pathogenic plate were grown in triplicate and the zone of inhibition was measured in triplicate. The raw Ciprofloxacin, Amoxicillin and Ampicilin powder of 250mg were dissolved in 20ml water and vortex repeatedly with slight heat. The antibiotics solutions were kept in refrigerator. From this stock, 20µl of solution were loaded to sterile discs made up of sterile filter paper and air dried. After drying the antibiotic disc were placed into pathogenic agar plates to test susceptibility. After incubation for 24 hours at 30 C a clear zone around a disc were the evidence of antimicrobial activity. The diameters of the zones of inhibition were measured in millimetres. Each test was prepared in triplicate and the zones of inhibition were measured. The triplicate samples were analysed for mean and standard deviation. The results are expressed as mean and standard deviation.

Results

The crude extracts of both seaweeds species possess antibacterial activity. *Chaetomorpha antennina* DMSO crude extract showed activity against all the three studied pathogens but its crude acetone extract have maximum inhibition of 10mm followed by chloroform (8.5mm) and dimethyl Sulphoxide (8mm) but its crude extracts had minimum activity against *Klebsiella pneumoniae* (0.2mm) with respect to positive control. *Amphiroa fragilissima* have minimum activity against all the three pathogens. In case of *Amphiroa fragilissima* DMSO extract had activity against all three pathogens but had maximum activity (0.7mm) against *Pseudomonas aeruginosa* followed by chloroform extract (0.6mm). The extracts were scanned with UV-Vis Spectrophometer (300nm -400nm) which developed peaks in various range of wavelengths indicating the presence of phytochemical, secondary metabolites and pigments.

Discussion

The variation in antibacterial activity may be due to the method of extraction, the type of solvents, season and location

Table-1. *The zone of inhibition of* Chaetomorpha antennina

Human pathogens	*Chaetomorpha antennina* extract, Diameter of inhibiting zone in mm (mean ± SD)					
	Water	DMSO	Ethyl acetate	Chloroform	Acetone	Ethanol
Pseudomonas aeruginosa	6.5±0.7	8±0.2	7.5±0.2	8.5±1.3	10±0.56	ND
Klebsiella pneumoniae	0.2±0.0	0.4±0.1	ND	ND	ND	ND
Escherichia coli	ND	2.2±0.0	ND	ND	ND	ND

Human pathogens	Positive Control		
	C1	A1	A2
Pseudomonas aeruginosa	6.70± 0.34	7.2±0.39	6.5±0.45
Klebsiella pneumoniae	2.56±0.56	0.15±0.10	ND
Escherichia coli	1.25±0.20	0.6±0.11	0.55±0.05

C1- Ciprofloxacin, A1- Amoxicillin, A2- Ampicilin

Table-2. *The zone of inhibition of* Amphiroa fragilissima

Human pathogens	*Amphiroa fragilissima* extract, diameter of inhibiting zone in mm (mean ± SD)					
	Water	DMSO	Ethyl acetate	Chloroform	Acetone	Ethanol
Pseudomonas aeruginosa	0.5±0.09	0.7±0.10	0.275±0.03	0.6±0.07	0.1±0.05	ND
Klebsiella pneumoniae	0.15±0.0	0.22±0.0	0.15±0.02	0.23±0.04	0.06±0.0	ND
Escherichia coli	0.16±0.0	0.2±0.02	0.14±0.01	ND	0.15±0.0	ND

Human pathogens	Positive Control		
	C1	A1	A2
Pseudomonas aeruginosa	ND	0.05±0.02	ND
Klebsiella pneumoniae	0.01±0.01	ND	ND
Escherichia coli	ND	ND	ND

C1- Ciprofloxacin, A1- Amoxicillin, A2- Ampicilin

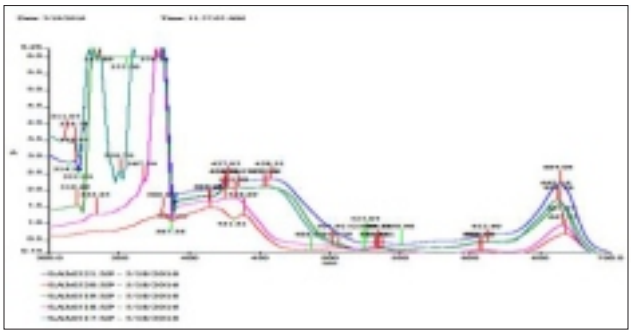

Fig.4. UV-Vis Spectrophometric analysis of *Chaetomorpha antennina* extracts: SAM-521:- DMSO, SAM-520:- Chloroform, SAM-519:- Ethanol, SAM-518: - Ethyl acetate, SAM-517:- Acetone

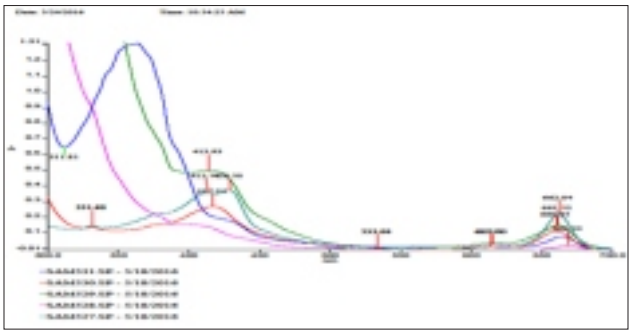

Fig. 5. UV-Vis Spectrophometric analysis of *Amphiroa fragilissima* extracts: SAM-521:- DMSO, SAM-520:- Chloroform, SAM-519:- Ethanol, SAM-518:- Ethyl acetate, SAM-517:- Acetone

from where samples were collected. *Chaetomorpha antennina* crude DMSO extract had maximum antibacterial activity for all three pathogens but the crude acetone extract had maximum activity which showed inhibition zone of 10mm, followed by crude chloroform extract (8.5mm) and DMSO extract (8mm) against *Pseudomonas aeruginosa* with respect to positive control. *Amphiroa fragilissima* showed minimum activity against all the three pathogens. In case of *Amphiroa fragilissima* DMSO extract had activity against all three pathogens but had maximum activity (0.7mm) against *Pseudomonas aeruginosa* followed by chloroform extract (0.6mm). UV-Vis Spectrophotometric analysis of extracts of *Chaetomorpha antennina* showed peaks between 660 nm to 670 nm for all extract as well as 427nm, 450nm. The maximum peaks developed at 335nm, 355nm and 376nm. For *Amphiroa anceps*, all extracts had peaks at 662 to 669 nm but DMSO and chloroform extract had a high peak at 300nm to 340nm. The peaks indicate the presence of various phytochemical, secondary metabolites and pigments. Finally, it can be assumed from this investigation that algal crude extract showed better antibacterial activity to human pathogens than the market available antibiotic. So, they are the potential sources of bio-active compounds for natural antibiotics.

Acknowledgements

The authors are thankful to Dr. S.T. Soumosundaram, Associate Professor, CAS in Marine Biology, Annamalai University for presnting instrumental facilities in his laboratory to conduct this experiment and the Head of the Department and staffs of Department of Microbiology, Rajah Muthiah Medical College & Hospital for provide the human pathogens. We also thank the higher authorities of Annamalai University.

References

Bauer, A. W., T.M. Sherris, W.H.M. Kirbay, M. Turk 1966. Antibiotic susceptibility of testing by standardized single disc method. *Am. J. Clin. Pathol.,* 45: 493-496.

Shimaa, M., El Shafay, S. Samh, M. Ali, M. ElSheekh, 2016. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. *The Egyptian Journal of Aquatic Research,* 42(1) : 65–74.

Harder, R. 1917. Ernahrungphysiologische untersuchughen a Cyanophyceen, Hauptsachlich am endophytischen Nostoc punctiformie. *Z. Bot.,* 9: 145.