Seaweed Res. Utiln., 24 (1) : 95 - 99, 2002

Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens

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ABSTRACT

Fifty five seaweed extracts belonging to 11 species of seaweeds were tested against post operative infectious drug resistant bacteria *viz., E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogens, Staphylococcus aureus.* Among the seaweed extracts, the acetone extracts of *Caulerpa cupressoides* shows maximum inhibtory activity against *E. coli* and propanol extracts of *Gracilaria edulis* shows maximum inhibitory effect against *K. pneumoniae.* Acetone extracts of *Padina tetrastromatica* and *Laurencia cruciata* show maximum inhibitory activity against *P. aeruginosa,* butanol extracts of *Hypnea musciformis, Caulerpa cupressoides* and *Chaetomorpha linoides* show maximum inhibitory effect against *S. aureus.*

Introduction

Hospital associated diseases are called nosocomial infections. It develops in the patient during stay in the hospital. A recent study indicates that 5 to 19% of the patients in acute care hospitals develop a nosocomial disease. Bacteria are the leading cause of nosocomial disease and viruses are a distant second. Occasionally fungi cause disease but rarely protozoa are involed. Mostly nosocomial diseases are caused by gram negative bacilli like *E. coli*. The use of therapeutic and diagnostic equipment (such as intravenous and urinary catheters), surgical procedure and transplantation has increased the risk of nosocomial diseases. Increased antibiotic therapy leads to the development of drug resistance against wide spectrum of antibiotics. Hence, this study has been undertaken to find out the new drugs from marine living resources.

Multiple drug resistance organisms are becoming common causes for infections in acute and long term care units in hospitals (Emori and Gaynes, 1993). The emergence of the resistant bacteria has created a major concern and an urgent need for new antibacterial agents (American society of Microbiology, 1995; Davis 1994; Spratt, 1994). Marine environment is an exceptional reservoir of biologically active natural products, many

of which exhibit structural features not been found in terrestrial natural products (Ireland *et. al.*, 1988). Marine natural products, the secondary or non-primary metabolites produced by living organisms, have been exploited by people for a variety of purposes including their use as food, fragrance, pigments, insecticides and medicines. Each year, an increasing number of novel marine metabolites are reported indicating that the marine environment is likely to continue to be a prolific source of new natural products, for many years to come. Approximately 2500 new metabolites were reported from a variety of marine organisms during the decade from 1977-1987 (Ireland *et. al.*, 1993). Because of the diversity of marine organisms in various habitats, marine natural products encompass a wide variety of chemical classes including terpenes, shikimates, polyketides, acetogenins, peptides and alkaloids of varying structures and multitude of compounds of mixed biosynthesis.

Materials and Methods

Collection of seaweed samples

Fresh seaweed samples of *Caulerpa cupressoides, Chaetomorpha linoides, Enteromorpha intestinalis, Gracilaria edulis, Colpomenia sinuosa, Hypnea musciformis, Laurencia cruciata, Padina boergesenii, P. tetrastromatica, Sargassum wightii* and *Ulva lactuca* were collected from the south west coast of India. Healthy and well grown plants were collected and cleaned with seawater and then freshwater to remove all epiphytes etc. and dried in room temperature. The dried samples were powdered for the extraction of antimicrobial compounds.

Isolation of drug resistant POI pathogens

Pus samples were collected from 5 male and 5 female patients from post operative wounds by touching the infected area with a sterile swab (antiseptic agent was not applied before taking the specimen). The collected swabs were transferred immediately in a sterile test tube and then into a transport medium. Two swabs were collected of which one was used for direct examination and another swab was inoculated into the solid media (Blood agar and Mac conkey's agar) and incubated at 35°C for 18-24 hrs. After incubation, the morphology of the colonies were observed and identified as *E. coli, K. pneumoniae, P. aeruginosa, S. pyogens, S. aureus* based on Bergey's manual of determinative bacteriology (Holt *et. al.*, 1994). Identified strains were checked for antibiotic resistance by following the method of Bauer *et. al.* (1996).

Extraction and bioassay of antimicrobial compounds

Dried powder of (50 g) seaweed samples were immersed in various solvents (Acetone, n - Butanol, propanol, water and Benzene). Percolation were carried out three times and kept for evaporation. The dried extract powder was redissolved in the respective solvents and used for the bioassay. Twenty milligram of evaporated extracts were impregnated into a sterile filter paper disc (5 mm diameter). The impregnated extracts and commercial antibiotic discs (30 antibiotics) were placed on seeded agar plates and kept it for incubation. After 24 hrs of incubation, the zone of inhibition of the bacterial species were measured by using graduated scale and expressed as millimeter in diameter.

Results and Discussion

There are five bacterial species isolated from post operative infectious wounds and identified as *E. coli, K. pneumoniae, P. aeruginosa, S. pyogens, S. aureus.* These strains were screened for antibiotic resistance with 30 antibiotics. The resistant antibiotics against each bacterial strain are presented in Table 1. Eleven crude seaweed extracts were

Table 1. *List of antibiotics resistant to the five bacterial pathogens*

Name of the Antibiotics	Name of the bacterial pathogens				
	E.c	K.p	P.a	S.p	S.a
Ceftazidine	R	R	R	R	R
Lincomycin	R	R	R	R	S
Vancomycin	R	S	R	S	S
Co-Trimazine	R	R	R	R	R
Nitrofurantoin	R	R	R	R	S
Streptomycin	R	R	S	S	S
Cephalothin	R	R	R	R	S
Clindamycin	R	S	R	R	R
Cloxacillin	R	R	S	R	R
Oxytetracycline	R	R	R	S	S
Penicillin - G	R	R	R	S	S
Co-Trimoxazole	R	R	R	S	S
Erythromycin	R	S	R	R	R
Amoxycillin	R	S	S	S	S
Cephalexin	R	R	S	S	S
Cephaloridine	R	S	S	S	S
Methicillin	R	S	S	S	S
Oleandomycin	R	S	S	S	S
Nallidixic acid	R	S	S	S	S
Ampicillin	R	R	R	S	S
Colistin	R	S	S	S	S
Oxacillin	R	S	S	S	S
Bacitracin	R	S	R	S	S
Polymycin	R	S	S	S	S
Neomycin	R	S	S	R	S
Cephalotoxine	S	R	S	S	S
Carbenicillin	S	R	S	S	S
Gentamycin	S	R	S	S	S
Norfloxacin	S	R	S	S	S
Furazolidone	S	S	S	R	S

E.c - Escherichia coli, K.p - Klebsiella pneumoniae, P.a -Pseudomonas aeruginosa, S.p - Streptococcus pyogens, S.a - Staphylococcus aureus

R - Resistance; S - Sensitive

tested against drug resistant post operative infectious pathogens (Table 2). Among the seaweed extracts, the acetone extract of *Caulerpa cupressoides* gives maximum inhibitory activity (9 mm) against the bacterium *E. coli.* The propanol extract of *Gracilaria edulis* gives maximum inhibitory activity (9 mm) against the bacterial pathogen *K. pneumoniae.* The acetone extract of *Padina tetrastromatica* and *Laurencia cruciata* gives maximum inhibitory activity (10 mm) against *P. aeruginosa.* The butanol extract of *Hypnea musciformis, Caulerpa cupressoides* and *Chaetomorpha linoides* gives the maximum inhibitory activity (8 mm) against *S. pyogens.* The butanol extract of *Padina tetrastromatica* gives the maximum inhibitory activity (10 mm) against *S. aureus.* Among the five species of pathogens, the inhibitory activity was in the series of *Pseudomonas aeruginosa, S. aureus, E. coli, K. pneumoniae* and *S. pyogens.*

The present study reveals the poor activity of water extract in all the test plant samples. Among the organic solvent extracts, the butanol, propanol and acetone extracts and not of benzene were highly effective against all post operative pathogens. Best and higher activity type was found in *Padina tetrastromatica* and *Laurencia cruciata* against *Pseudomonas aeruginosa* followed by *Padina boergesenii* extract against *S. aureus.* Pesando and Caram (1984) reported that *E. coli* was more sensitive to *Laurencia obtusa.* Issac and Hedge (1987) found that *E. coli* was more sensitive to *Sargassum johnstonii.* Glombitza (1970) suggests that dimethyl sulphide and acrylic acid from seaweeds has antimicrobial activity. Pratt *et. al.* (1951), observed that *Spirogyra* sp. was known to reduce typhoid,

Table 2. *Sensitivity pattern of seaweed extract against five species of multiple drug resistant nosocomial infectious pathogenic bacteria.*

Name of the seaweed	Solvent used	Zone of inhibition in mm					Name of the seaweed	Solvent used	Zone of inhibition in mm				
		E.c	K.p	P.a	S.p	S.a			E.c	K.p	P.a	S.p	S.a
Padina tetrastromatica	Benzene	NS	NS	6	6	6	*Chaetomorpha linoides*	Benzene	7	6	6	NS	8
	Butanol	7	6	NS	NS	9		Butanol	7	8	8	8	6
	Propanol	8	8	7	6	8		Propanol	6	8	6	6	NS
	Acetone	NS	NS	10	7	6		Acetone	NS	NS	5	6	NS
	Water	6	NS	NS	NS	NS		Water	6	NS	NS	NS	NS
Hypnea musciformis	Benzene	6	6	6	7	7	*Ulva lactuca*	Benzene	6	6	6	6	6
	Butanol	8	5	6	8	8		Butanol	6	7	NS	NS	8
	Propanol	7	6	6	7	6		Propanol	6	6	6	NS	7
	Acetone	NS	NS	NS	NS	NS		Acetone	NS	NS	8	NS	NS
	Water	6	NS	NS	10	NS		Water	NS	NS	NS	NS	NS
Laurencia cruciata	Benzene	6	7	5	5	5	*Sargassum wightii*	Benzene	6	NS	6	6	6
	Butanol	7	5	7	NS	6		Butanol	8	8	7	NS	9
	Propanol	6	NS	NS	6	5		Propanol	6	6	6	6	5
	Acetone	8	NS	10	7	NS		Acetone	NS	NS	NS	NS	NS
	Water	6	7	NS	NS	NS		Water	NS	NS	NS	NS	NS
Caulerpa cupressoides	Benzene	6	6	5	6	6	*Gracilaria edulis*	Benzene	6	6	6	6	6
	Butanol	7	NS	7	NS	6		Butanol	8	8	7	7	7
	Propanol	7	NS	8	7	6		Propanol	5	9	6	7	6
	Acetone	9	6	5	8	7		Acetone	NS	NS	5	6	5
	Water	6	6	NS	NS	NS		Water	NS	NS	NS	NS	NS
Enteromorpha intestinalis	Benzene	6	NS	6	6	6	*Colpomenia sinuosa*	Benzene	7	6	6	NS	NS
	Butanol	7	7	6	7	6		Butanol	8	8	8	7	6
	Propanol	6	NS	7	7	7		Propanol	6	NS	7	7	6
	Acetone	NS	NS	NS	NS	NS		Acetone	NS	NS	5	NS	NS
	Water	6	10	NS	NS	NS		Water	NS	NS	NS	NS	NS
Padina boergesenii	Benzene	6	6	6	NS	NS							
	Butanol	8	7	NS	NS	10							
	Propanol	6	6	7	7	7							
	Acetone	7	NS	5	NS	NS							
	Water	5	NS	NS	NS	NS							

E.c - Escherichia coli, K.p - Klebsiella pneumoniae, P.a Pseudomonas aeruginosa, S.p - Steptococcus pyogens, S.a - Staphylococcus aureus NS - No Sensitivity

cholera, dysentry. Padmini Sreenivasa Rao and Karmarkar (1986) reported that ethanol extract of *S. johnstonii, Enteromorpha* sp and *Corallina officinalis* showed high activity against *S. aureus* than the extracts of acetone and n - butanol. Xixu and Lee (2001) reported that the plant flavanoids highly inhibited the growth of multiple drug resistant *Staphylococcus aureus* and *Burkholderia cepacia.* To our knowledge this is the first report from India of bioactive compounds effective against the multiple drug resistant bacteria.

Acknowledgements

The authors are grateful to the authorities of M. S. University, Tirunelveli for providing facilities and to the Tamilnadu State Council for Science and Technology for sanctioning the Student Project.

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