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Antimicrobial Activity of Seaweeds Against Multi Drug Resistant Strains

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Abstract: Nowadays, emergence of bacterial resistance poses a significant clinical problem. Hence, the aim of this study was to describe the current susceptibility patterns of Multi Drugs Resistant (MDR) strains of Urinary Tract Infections (UTI) isolates to current chemotherapeutic agents, as well as to find out antimicrobial characteristics in the extract of seaweeds against MDR. The extract of *Padina tetrastromatica, Stocheospermum marginatum* and *Grateloupia lithophila* exhibited strong activity against Non-MDR strains, whereas, the extract of *Grateloupia lithophila* only exhibited moderate activity against MDR strains. The extract of *Caulerpa sp., Gracilaria corticata* and *Valaniopsis paachanima* exhibited week antimicrobial activity. The extract of *Grateloupia lithophila* inhibited the growth of both MDR and Non-MDR *Staphylococcus aureus*. Moreover, extract of *Grateloupia lithophila* inhibited the growth of Non-MDR *E. coli, Pseudomonas aeruginosa* and *Klebsiella*. From our findings, the most potent antimicrobial seaweed extract was *Grateloupia lithophila* which showed maximum inhibitory activity against MDR and Non-MDR *Staphylococcus aureus*. This study recommends extracts of *Grateloupia lithophila* can also be used as antibacterial substance for treating multidrug resistant microbes causing acquired infections.

Key words: Seaweed, *Grateloupia lithophila*, multidrug resistant, susceptibility *Staphylococcus aureus*

INTRODUCTION

Nowadays, drug resistance is a problem in treating infectious diseases like malaria, Tuberculosis (TB), diarrheal diseases and urinary tract infections. A suggested by Manikandan *et al*. (2011) the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance which became a major health problem worldwide. In the past decade, many kinds of resistant strains have been discovered. For example, methicillin resistant *Staphylococcus aureus* (MRSA) (Wagenlehner and Naber, 2004), multidrug resistant *Pseudomonas aeruginosa* (Linuma, 2007) and *Serratia marcescens* (Kim *et al*., 2006), Vancomycin Resistant Enterococci (VRE) (Gold, 2001) and Extended Spectrum Beta Lactamase (ESBL) resistant enterococci (Bhattacharya, 2006) have been reported previously. Drug resistance in pathogens is a serious medical problem, because of fast rise and spread of mutant strains that are insusceptible to medical treatment.

UTIs are the most common of the infectious diseases. Worldwide, about 150 million people are diagnosed with UTI every year (Gonzalez and Schaeffer, 1999). Confronting difficulties of treatment of UTIs is seen with increased frequency. The antimicrobial susceptibilities of the pathogens are predictable because of the widespread pathogens of UTIs have been found resistant to most chemotherapeutic agents (Okeke *et al*., 2009). At this juncture, it is of paramount importance to find alternative drugs to combat MDR strains. The past records of rapid and widespread emergence of resistance to newly introduced antimicrobial agents indicate that even new families of antimicrobial agents will have short life expectancy (Coates *et al*., 2002). For this reason, researchers are increasingly turning their attention to isolate better drugs for treating MDR strains from marine sources. The marine organisms may offer an extremely rich resource of structurally novel and biologically active metabolites. So far, many chemically unique compounds of marine origin, with different biological activities, have been isolated and a number of them are under investigation or are being developed as new pharmaceuticals (Faulkner, 2000; Da-Rocha *et al*., 2001).

The importance of marine resource has recently been emphasized due to the increasing demand for them as medicinal products like antibiotics (Hoppe, 1979) such as antibacterial, antifungal (Tang *et al*., 2002), antiviral (Serkedjieva, 2004), antitumour, antioxidant agents and medicinal value food (Madhusudan *et al*., 2011; Fakoya *et al*., 2011). Much previous research has studied the biological effects of natural marine products.

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However, only a few studies have examined the biological potential of the marine algae (seaweeds) (Eluvakkal *et al*., 2010).Thus, seaweeds are considered as a source of bioactive compounds and produce a great variety of secondary metabolites exhibiting broad spectrum of biological activities and associated microorganism also produce bioactive compound (Ramasamy and Kumar, 2009). Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al*., 2003; Kim *et al*., 2009). According to Khotimchenko *et al*. (2002) seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world. The aim of the present study was to describe the current susceptibility patterns of UTI isolates to current chemotherapeutic agents, as well as to find out antimicrobial characteristics in the methanol extracts of seaweeds against Multi Drugs Resistant (MDR) strains.

MATERIALS AND METHODS

Isolation of multidrug resistant strains: Urine samples were collected from community acquired UTI patient in the month of August 2009. Isolated strains from the samples were identified based on staining and biochemical tests. From this, multidrug resistant strains were isolated by conducting antimicrobial susceptibility test of Kirby *et al*. (1957). Bauer disk diffusion method (1957) following the definition of the National Committee of Clinical Laboratory Standards (NCCLS 46) against common antimicrobial agents such as Ampicillin, Cephalexin, Ciprofloxacin, Cotrimoxazole, Gentamycin, Nalidixic acid, Streptomycin and Vancomycin (Himedia Labs, Mumbai, India).

Seaweed extraction and antimicrobial assays: Seaweeds were collected from intertidal rockyshore region in Pudhumadam in the Gulf of Mannar, India during August 2010 and identified using keys provided according to Ganesapandian and Kumaraguru (2008). After air dried, samples were homogenized. Each 25 g of homogenized seaweeds were suspended in 100 mL of methanol for 24 h and the extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the results were the mean of three replicates (Livermore and Brown, 2001).

RESULTS

Table 1 gives the multi-drug resistance profile of various isolates to the routinely used antibiotics. Four UTI strains were isolated and tested for susceptibility against some antibiotics, many of antibiotics showed resistance to the MDR strains. Among this *E. coli* and *P. aeruginosa* were highly resistant to most of the antibiotics, whereas *S. aureus* exhibited sensitivity to Cephalexin, Ciprofloxacin, Cotrimoxazole and Streptomycin.

Antibacterial activity exhibited in most of the methonol extracts of seaweeds was summarized in Table 2. Extract of *Padina tetrastromatica* and *Grateloupia lithophila* showed good inhibiting activity against *S. aureus*. The extract of *Grateloupia lithophila* also showed good inhibiting activity against *E. coli*. Where as, extract of *Acanthophora specifera, Dictyota dichotoma, Gracilaria corticata* and *Chaetomorpha antennina* showed weak inhibiting activity against most of the non-MDR strains. *Caulerpa peltata* and *Caulerpa scalpelliformis* did not show any inhibiting activity against all non-MDR strains.

Table 1: Susceptibility pattern of Antibiotic against UTI isolates

Antibiotic	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Ampicillin (10 µg)	R	R	R	R
Cephalexin (10 µg)	R	S	S	S
Ciprofloxacin (10 µg)	R	S	R	S
Cotrimoxazole (10 µg)	R	R	S	S
Gentamycin (10 µg)	S	S	R	R
Nalidixic acid (30 µg)	R	R	R	R
Streptomycin (10 µg)	S	R	S	S
Vancomycin (10 µg)	R	S	R	R

R: Resistant, S: Sensitive

Table 2: Susceptibility pattern of crude methanol seaweeds extracts against Non-MDR

Seaweeds	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Chaetomorpha antennina	-	+	+	++
Caulerpa peltata	-	-	-	-
Caulerpa scalpelliformis	-	-	-	-
Valauiopsis paachanima	-	-	+	-
Dictyota dichotoma	+	-	+	+
Padina tetrastromatica	-	++	++	+++
Stoechospermum marginatum	++	-	+	+++
Acanthophora specifera	+	-	+	-
Grateloupia lithophila	+++	++	++	+++
Gracilaria corticata	-	-	-	+

-: No activity, +: Weak activity (1-5mm halo), ++: Moderate activity (6-10mm halo), +++: Good activity (10-15-mm halo)

Table 3: Susceptibility pattern of crude methanol seaweeds extracts against MDR strain

Seaweeds	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Chaetomorpha antennina	-	-	-	+
Caulerpa peltata	-	-	-	-
Caulerpa scalpelliformis	-	-	-	-
Valaviopsis paachanima	-	-	-	-
Dictyota dichotoma	-	-	+	-
Padina tetrastromatica	-	+	-	+
Stoechospermum marginatum	-	-	-	+
Acanthophora specifera	-	-	-	-
Grateloupia lithophila	+	-	++	++
Gracilaria corticata	-	-	-	-

-: No activity, +: Weak activity (1-5mm halo), ++: Moderate activity (6-10mm halo), +++: Good activity (10-15-mm halo)

Extract of *Grateloupia lithophila* showed moderate inhibiting activity against MDR strains of *S. aureus* and *Pseudomonas* (Table 3). UTIs pathogenic *E. coli* was resistant to most of the extracts except *Grateloupia lithophila*. The extract of *Chaetomorpha antennina, Dictyota dichotoma, Padina tetrastromatica* and *Stoechospermum marginatum* showed weak inhibiting activity against the MDR isolates. Whereas, methanol extracts of *Caulerpa peltata, Caulerpa scalpelliformis, Acanthophora specifera* and *Gracilaria corticata* did not show any inhibiting activity against MDR isolates.

DISCUSSION

Drug resistance is one of nature's never ending process by which the organisms develop tolerance to new environmental conditions. It may be due to a pre-existing factor in the organisms or due to acquired factor(s). Rella and Haas (1982) first reported nalidixic acid resistant *P. aeruginosa* of UTI which showed resistance to β-lactam antibiotics. A significant increase in resistance of UTI pathogenic strains to Ampicillin has been found worldwide (Sahm *et al*., 2001). Therefore these agents should not be recommended for first line empirical treatment of UTI (Warren *et al*., 1999). Nalidixic acid has been recommended as drug of choice for the treatment of uncomplicated UTIs (Onifade *et al*., 2005; Akortha and Ibadin, 2008) because of its activity against several different types of Gram negative bacteria such as *E. coli*, *Klebsiella* sp. etc. The findings of this study coincide with the findings of Shittu and Mandere (1999) that *S. aureus* strains were highly resistant to naladixic acid. All the isolates in this study showed resistance to at least 5-7 different antibiotics, indicating the presence of strong selective pressures from the antibiotics in the community. Brown *et al*. (2003) have reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates and suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption as previously suggested by Nwanze *et al*. (2007).

In this study, a multidrug resistance strain was found which are resistant to most of the antimicrobial agent tested. This reflected the fact that ampicillin, tetracyclin and streptomycin were the most commonly prescribed antibiotics in the hospitals even before the results of urine analyses and also the most easily available in the market without prescription because they are also cheap in terms of cost. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria. Higher resistant strains were reported in the USA for ampicillin and cotrimoxazole (Sahm *et al*., 2001), where as few ciprofloxacin resistant strains were found in other countries (Diekema *et al*., 2004; Mohammadi *et al*., 2010). This study also noticed ciprofloxacin-resistant *E. coli* from UTIs. Ciprofloxacin as an option for therapy to UTIs has been considered, since its multiple mechanisms of action seem to have enabled it to retain potent activity against *E. coli*. Ciprofloxacin has high level of activity against UTI isolates of *E. coli* compared with other commonly used agents, such as Ampicillin and SXT (Gupta *et al*., 1999).

Gonzalez del val *et al*. (2001) reported that the methanolic extract of *Padina* showed antibacterial activity only against *B. subtilis*. However, in the present study the methanol extract of *P. tetrastromatica* inhibited the growth of *K. pneumoniae* and *S. aureus* and it was unable to inhibit the growth of *E. coli*. Similar to this study Tuney *et al*. (2006) reported acetone, methanol and diethyl ether extracts of *Padina* had no antibacterial activity but ethanol extract of *Padina* had weak activity against *E. faecalis*, *P. aeruginosa* and *E. coli*. In the present study, it was observed that *E. coli* was resistant to methanol extract of all algae except *Grateloupia lithophila* and *Stoechospermum marginatum*. Salvador *et al*. (2007) also reported that *E. coli* was resistant to the extracts of *Dictyota dichotoma*, *Malopteris filicine*, *Cladostephus spongiosus*, *F. verticillatus and Ulva rigida*. It may be due to the methanol used as an organic solvent for extracting compounds providing a higher efficiency of antimicrobial activity.

The current study revealed that the inhibitory activity of extract of *Grateloupia lithophila* showed higher in the MDR isolates of *Pseudomonas* and *S. aureus* and Non-MDR isolates of *E. coli, Klebsiella* and *S. aureus* than the inhibitory activity exhibited by vancomycin and gentamycin disc. The MDR isolate *E. coli,* was also inhibited by the same extract but had smaller inhibition zone with 1mm which is less than the inhibition activity of vancomycin disc. The methanol extract of *Grateloupia lithophila* exhibited intense activity against both MDR and non-MDR strains. Kandhasamy and Arunachalam (2008) reported that methanolic extract of *Gracilaria* and *Caulerpa* inhibited the growth of all pathogenic bacteria except *E. coli*. Rao and Parekh (1981) reported extracts of *Enteromorpha intestinalis* and *G. corticata* have antibacterial activity. In contrast to these investigations, our study showed that *Caulerpa* have less activity where as *G. corticata* did not show antibacterial activity as reported by Hornsey and Hide (1985).

CONCLUSION

These results indicate the possibility that different quantities of active secondary products were synthesized under different conditions. Differences between the results of the present investigation and results of other studies may be due to the production of bioactive compounds related to e seasonal variations, extraction methods, organic solvents used for extraction of bioactive compounds and different bioassay methods. Finally it can be concluded that extracts of algal species used in the present investigation showed better antibacterial activity against MDR pathogens. Seaweeds are the potential sources of bioactive compounds and should be investigated for natural antibiotics. This study recommends seaweed extracts as antibacterial substance for treating multidrug resistant microbes causing acquired infections.

REFERENCES

Akortha, E.E. and O.K. Ibadin, 2008. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with Urinary Tract Infection (UTI) in UBTH Benin City, Nigeria. Afr. J. Biotechnol., 7: 1637-1640.

Bhattacharya, S., 2006. ESBL-from petri dish to the patient. Indian J. Med. Microbiol., 24: 20-24.

Brown, J.R., D. Gentry, J.A. Becker, K. Ingraham, D.J. Holmes and M.J. Stanhope, 2003. Horizontal gene transfer of drug-resistant amino-acyl-transfer-RNA synthetases of anthrax and Gram-positive. EMBO Rep., 4: 692-698.

Coates, A., Y. Hu, R. Bax and C. Page, 2002. The future challenges facing the developement of new antimicrobial drugs. Nat. Rev. Drug Discov., 1: 895-910.

Da-Rocha, A.B., R.M. Lopes and G. Schwartsmann, 2001. Natural products in anticancer therapy. Curr. Opin. Pharmacol., 1: 364-369.

Diekema, D.J., B.J. BootsMiller, T.E. Vaughn, R.F. Woolson and J.W. Yankey *et al*., 2004. Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clin. Infect. Dis., 38: 78-85.

Eluvakkal, T., S.R. Sivakumar and K. Arunkumar, 2010. Fucoidan in some Indian brown seaweeds found along the coast gulf of mannar. Int. J. Bot., 6: 176-181.

Fakoya, K.A., F.G. Owodeinde, S.L. Akintola, M.A. Adewolu, M.A. Abass and P.E. Ndimele, 2011. An exposition on potential seaweed resources for exploitation, culture and utilization in West Africa: A case study of Nigeria. J. Fish. Aquat. Sci., 6: 37-47.

Faulkner, D.J., 2000. Highlights of marine natural products chemistry (1972-1999). Nat. Prod. Rep., 17: 1-6.

Ganesapandian, S. and A.K. Kumaraguru, 2008. Seaweeds resource in the intertidal and subtidal regions of Pudumadam, Gulf of Mannar. Seaweed Res. Utilization, 30: 97-105.

Gold, H.S., 2001. Vancomycin-resistant enterococci: Mechanisms and clinical observations. Clin. Infect. Dis., 33: 210-219.

Gonzalez del val, A., G. Platas, A. Basilio, A. Cabello and J. Gorrochategui *et al*., 2001. Screening of antimicro bial activities in red, green and brown macro algae from Gran Canaria (Canary Islands, Spain). Int. Microbiol., 4: 35-40.

Gonzalez, C.M. and A.J. Schaeffer, 1999. Treatment of urinary tract infection: What's old, what's new and what works. World. J. Urol., 6: 372-382.

Gupta, K., D. Scholes and W.E. Stamm, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. J. Am. Med. Assoc., 281: 736-738.

Hoppe, H.A., 1979. Marine Algae and their Products and Constituents in Pharmacy. In: Marine Algae in Pharmaceutical Science, Hoppe, H.A., T. Levring and Y. Tanaka (Eds.). Water de Gruyter, Berlin, pp: 25-119.

Hornsey, I.S. and D. Hide, 1985. The production of antimicrobial compounds by British marine algae. IV variation of antimicrobial activity with algal generation. Eur. J. Phycol., 20: 21-25.

Kandhasamy, M. and K.D. Arunachalam, 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southest coast of India. Afr. J. Biotechnol., 7: 1958-1961.

Khotimchenko, S.V., V.E. Vaskovsky and T.V. Titlyanavo, 2002. Fatty acids of marine algae from the Pacific coast of North California. Bot. Marina, 45: 17-22.

Kim, B.N., S.I. Choi and N.H. Ryoo, 2006. Three-year follow-up of an outbreak of *Serratia marcescens* bacteriuria in a neurosurgical intensive care unit. J. Korean Med. Sci., 21: 973-978.

Kim, K.N., Y.M. Ham, J.Y. Moon, M.J. Kim and D.S. Kim *et al*., 2009. *In vitro* cytotoxic activity of *Sargassum thunbergii* and *Dictyopteris divaricata* (Jeju Seaweeds) on the HL-60 tumour cell line. Int. J. Pharmacol., 9: 298-306.

Kirby, W.M.M., G.M. Yoshihara, K.S. Sundsted and J.H. Warren, 1957. Clinical Usefulness of a Single Disc Method for Antibiotic Sensitivity Testing. Antibiotics Annual, Medical Encyclopaedia Inc., New York, pp: 892-897.

Linuma, Y., 2007. Infection control strategies for antimicrobial resistance. Nippon Rinsho, 65: 175-184.

Livermore, D.M. and D.F. Brown, 2001. Detection of beta-lactamase-mediated resistance. J. Antimicrob. Chemother., 48: 59-64.

Madhusudan, C., S. Manoj, K. Rahul and C.M. Rishi, 2011. Seaweeds: A diet with nutritional, medicinal and industrial value. Res. J. Med. Plant, 5: 153-157.

Manikandan, S., S. Ganesapandian, M. Singh and A.K. Kumaraguru, 2011. Emerging of multidrug resistance human pathogens from urinary tract infections. Curr. Res. Bacteriol., 4: 9-15.

Mohammadi, M., E. Ghasemi, H. Mokhayeri, Y. Pournia and H. Borou, 2010. Antimicrobial resistance patterns of *E.coli* detected from hospitalized urine Culture samples. Asian J. Biol. Sci., 3: 195-201.

Newman, D.J., G.M. Cragg and K.M. Snader, 2003. Natural products as source of new drugs over the period 1981-2000. J. Nat. Prod., 66: 1022-1037.

Nwanze, P.I., L.M. Nwaru, S. Oranusi, U. Dimkpa and M.U. Okwu *et al*., 2007. Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. Sci. Res. Essays., 2: 112-116.

Okeke, I.N., O.A. Aboderin, D.K. Byarugaba, K.K. Ojo and J.A. Opintan, 2009. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg. Infect. Dis., 13: 1640-1646.

Onifade, A.K., F.O. Omoya and D.V. Adegunloye, 2005. Incidence and control of urinary tract infections among pregnant women attending antenal clinics in government hospitals in Ondo State, Nigeria. J. Food Agric. Environ., 3: 37-38.

Ramasamy, M.S. and S.S. Kumar, 2009. Anti inflammatory, antinociceptive and central nervous system depressant activities of marine bacterial extracts. J. Pharmaco. Toxicol., 4: 152-159.

Rao, P.S. and K.S. Parekh, 1981. Antibacterial activity of Indian seaweed extracts. Bot. Marina, 24: 577-582.

Rella, M. and D. Haas, 1982. Resistance of *Pseudomonas aeruginosa* PAO to nalidixic acid and low levels of β-lactam antibiotics: Mapping of chromosomal genes. Antimicrob. Agents Chemother., 22: 242-249.

Sahm, D.F., C. Thornsberry, D.C. Mayfield, M.E. Jones and J.A. Karlowsky, 2001. Multidrug-resistant urinary tract isolates of *E. coli*: Prevalence and patients demographics in the United States in 2000. Antimicrob. Agents Chemother., 45: 1402-1406.

Salvador, N., A. Gomez Garreta, L. Lavelli and M.A.R. Siguan, 2007. Antimicrobial activity of Iberian macroalgae. Sci. Mar., 71: 101-114.

Serkedjieva, J., 2004. Antiviral activity of the red marine algae *Ceramium rubrum*. Phytother. Res., 18: 480-483.

Shittu, S.O. and M.U. Mandere, 1999. Asymotomatic bacteriuria in antenatal patients in A.U.TH Zaria. Trop. J. Obst. Gynea., 16: 41-41.

Tang, H.F., Y. Yang-Hua, X.S. Yao, Q.Z. Xu, S.Y. Zhang and H.W. Lin, 2002. Bioactive steroids from the brown algae *Sargassum carpophyllum*. J. Asian Nat. Prod. Res., 4: 95-105.

Tuney, I., B.H. Cadirci, D. Unal and A. Sukatar, 2006. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turk. J. Biol., 30: 171-175.

Wagenlehner, F.M. and K.G. Naber, 2004. New drugs for Gram-positive uropathogens. Int. J. Antimicrob. Agents., 24: S39-S43.

Warren, J.W., E. Abrutyn, J.R. Hebel, J.R. Johnson, A.J. Schaeffer and W.E. Stamm, 1999. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin. Infect. Dis., 29: 745-758.