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 tetrasremium, Stoechospermum 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 pachyana exhibited weak 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 last decade, many kinds of resistant strains have been discovered. For example, methicillin resistant Staphylococcus aureus (MRSA) (Wagenlehner and Naber, 2004), multidrug resistant Pseudomonas aeruginosa (Liruma, 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 insensitive 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 Schieffer, 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 (Serkedjiev, 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.
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, Gentamicin, 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, Indi during August 2010 and identified using keys provided according to Ganesapandian and Kumanaguru (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 methanol 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 pelata and Caulerpa scalpelliformis did not show any inhibiting activity against all non-MDR strains.

### Table 1: Susceptibility pattern of Antibiotic against UTI isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E. coli</th>
<th>K. pneumonia</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10 μg)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cephalexin (10 μg)</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin (10 μg)</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cotrimoxazole (10 μg)</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin (10 μg)</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nalidixic acid (30 μg)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Streptomycin (10 μg)</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Vancomycin (10 μg)</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>R: Resistant, S: Sensitive</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Seaweeds</th>
<th>E. coli</th>
<th>K. pneumonia</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetomorpha antennina</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Caulerpa pelata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caulerpa scalpelliformis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Valonia ocellata</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dictyota dichotoma</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Padina tetrastromatica</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Stoechospermum marginatum</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Acanthophora specifera</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Grateloupia lithophila</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Gracilaria corticata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- : 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 pelata, Caulerpa scapellariformis, Acanthophora specifera* and *Gracilariopsis 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 nalidix 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 Shitu 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 ciprofloxacins resistant strains were found in other countries (Diekema et al., 2004; Mohammadi et al., 2010). This study also noticed ciprofloxacins-resistant *E. coli* from UTIs. Ciprofloxacins 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*. Ciprofloxacins 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. coli, 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, Malaporteria filicina, Cladophora spongiosa, F. verticillatus* and *Ulua 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 activities 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 methanol 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 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


