Antibacterial activity of Ascidian phallusia arabica against human clinical isolates

Selva Prabhu A, G. Ananthan, S. Mohamed Hussain and T. Balasubramanian

ABSTRACT

Antibacterial activity of methanol and ethanol extracts of the ascidian, Phallusia arabica was evaluated by disc diffusion method against various human pathogen isolates. Two different concentrations (0.5 and 1 mg/ml) were analyzed. The crude methanol extract was more active exhibiting a broad-spectrum antibacterial activity than the ethanol extract against the pathogenic microbes tested. Maximum inhibition zone (12 mm) was observed against Pseudomonas aeruginosa in 1 mg ml⁻¹ concentration crude methanol extract. The inhibition zone was 3 mm in Escherichia coli for ethanol extract of 0.5 mg ml⁻¹ concentration. The ranges of inhibition zone in the tested extracts were lesser than the standard antibiotics used in all the strains. The MIC and MBC for methanolic extract tested in study inferred that the values range between 0.70-0.95 mg/ml and 0.85-1.1 mg/ml respectively.

Keywords: Antibacterial activity; Phallusia arabica; disc diffusion method; human pathogens.

INTRODUCTION

Ocean has a number of organisms which are having potent bioactive compounds and are currently used as medicine. A large proportion of natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and mollusks and some of them are currently used in clinical trials (Proksch et al., 2002). Ascidians are marine invertebrates which ranks second with promising the source of drugs (Azumi et al., 1990). Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection (Bergquist and Bedford. 1978). This mechanism has proved timely to be an alternative natural medicine to human beings. From tunicates (ascidian) Trididemnum solidum, was the first marine compound entered into human cancer clinical trial as a purified natural product (Carte, 1996), but was unsuccessful in further trials (Davidson, 1993). Already various ascidians such as Botryllus sp., and Didemnum sp. were reported for producing anti cancer drugs (Azumi et al., 1990). Halocyanine A, an antimicrobial substance was isolated from haemocytes of the solitary ascidians Halocynthia roretzi (Azumi et al., 1990). Such potential ascidians should to be explored for the pharmaceutical purpose. Tunicates have been reported as rich sources of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans (Davis & Bremner, 1999). These compounds are mainly comprised of various derivatives of alkaloids and peptides. There are few examples of marine derived compounds which have successfully reached the market as therapeutic drugs. A large group of low molecular weight natural compounds that exhibit
antimicrobial activity has been isolated from plants and animals during the past two decades. The evolution of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. The powers of marine organisms have been realized for thousands of years and their potential as producers of pharmaceutical products have been reviewed (Baker, 2004).

MATERIAL AND METHODS

Collection and preparation of samples

The ascidian, Phallusia arabica (Chordata: Asciidiacea: Enterogona: Asciidiidae) were collected during the low tide of the intertidal area at Thoothukudi, southeast coast of India, during July 2011. The collected samples were rinsed with sterile seawater to remove associated debris and salt. Ten gram of the sample was weighed and preserved separately in methanol, ethanol mixture (1:2) and brought to the laboratory.

Antibacterial assay

The bioassay was carried out using the agar disc diffusion method (Baur et al., 1966). Muller Hinton agar plates are prepared by pouring 15 ml of medium and allowed to solidify. The petri plates are swabbed with 24 h old culture of the four selected bacterial strains. The sterile paper discs were loaded with different solvents, concentration and allowed to dry thoroughly. Then the discs were placed over the plates and incubated for 24 h at 37°C.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was determined by the following procedure (Collins et al., 1995).

Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates, followed by standard method of Alade & Irobi (1993).

RESULTS AND DISCUSSION

The results of antibacterial activity of the crude methanol and ethanol extract of P. arabica against different gram negative clinical isolates are given in Table 1.

Table 1: Antibacterial activity of the crude methanol and ethanol extract of Phallusia arabica against different gram negative clinical isolates.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Methanol</th>
<th>Ethanol</th>
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<tr>
<td></td>
<td>0.5mg/ml</td>
<td>1mg/ml</td>
</tr>
<tr>
<td>E.coli</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>S.aureus</td>
<td>5</td>
<td>7</td>
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</table>

Methanol extract of 1 mg/ml concentration produced a maximum inhibition zone of 12 mm against P. aeruginosa and the minimum of 4 mm in E.coli. The corresponding zones of ethanol extract produced 10 mm and 3 mm against E. coli and P. aeruginosa respectively. Both extracts in two different concentrations showed minimum activity against E.coli, whereas minimum activity was observed in the same strain at a concentration of 0.5mg/ml. Both extracts showed a broad spectrum antibacterial activity against P. aeruginosa, followed by K. pneumoniae, S. aureas and E.coli in all the two concentrations. This observation is consistent with the findings of Ananthan et al. (2011) who reported that both methanol and ethyl acetate extract of P. nigra showed a broad spectrum of antibacterial activity against tested gram negative pathogens. Antibacterial activity of ascidian extracts increased with increasing concentrations. Antibacterial and cytotoxic activity has been previously reported from extracts of some tunicates (Thompson et al., 1985; Mohamed Hussain and Ananthan, 2009; Sivaperumal et al., 2010; Ananthan et al., 2011 b; Mohamed Hussain et al., 2011).

The results of the present study showed that highly potent antibacterial substance is found in P. arabica. Marine ascidians contain antibacterial agent which can be used in relevance to either antifouling technology or clinical pharmacology. Antibacterial activity of the crude extract of ascidian showed inhibitory activity against almost all the four human pathogen strains. The range of inhibiting distance of bacteria varied from 3-12 mm. In some ascidian species, peptides with antibiotic properties in vitro have been shown to have other biological effects such as protection against predation, digestion or prevention of surface epibiosis. There is a great scope for finding further novel antimicrobial compounds in the ascidian group, and further research is needed including biochemical and seasonal changes in biologically active peptides. It was concluded from the study that methanolic extract of P. arabica exhibits potential antibacterial property. Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates. After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of extract that does not yield any colony growth on the solid medium after the incubation period was regarded as minimum bactericidal concentration (MBC) (Alade & Irobi, 1995).

In this study we examined antibacterial activity of the crude methanol and ethanol extract of the test body of P. arabica, against gram negative strains and it was evident that the gram negative strains were more resistant. On the other hand, Ananthan et al. (2011) reported the maximum antibacterial activity of the crude methanol extracts of the test and mantle bodies of P. arabica against the isolated urinary tract pathogens Pseudomonas aeruginosa Martinez and Baquero, (2002) have reported that, some of these bacteria out rightly developed multi drug resistance to some antibiotics. Recently from a 3 year follow up study in USA, Dowzicky and Park (2008) reported that, UTI bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Present results showed moderate antibacterial activity against the multi drug resistant clinical
isolates by the test body of *P. arabica*. This could be attributed to the fact that the test body might contain secondary metabolites which inhibit the growth of bacteria. This view is consistent with the findings of Paul *et al.*, (2008) that the tunicates have the potential to yield novel compounds of ecological, chemical and also biomedical interest. Two new tyrosine derivatives, botryllamides K (1) and L (2), together with six known metabolites, isolated from Australian ascidian, *Aplidium alatum* were evaluated for their cytotoxicity towards the tumor cell lines, MCF-7 (breast), H460 (lung) and SF268 (central nervous system) (Depenbrock, *et al.*, 1998; Urdiales, *et al.*, 1996; Cardenas *et al.*, 2001). We conclude that, the continuing and overwhelming contribution of ascidian metabolites to the development of new pharmaceuticals are clearly evident and need to be explored. Antibacterial compounds from natural resources would be the alternative to overcome the resistance problems. The minimum inhibitory concentrations (MICs) and Minimum bactericidal concentration (MBC) of the methanolic extract of *P. arabica* are shown in Table 2.

Table 2: MIC and MBC methanolic extract of Phallusia Arabica.

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
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<tr>
<td><em>E.coli</em></td>
<td>0.70</td>
<td>0.85</td>
</tr>
<tr>
<td><em>P.aeruginosa</em></td>
<td>0.95</td>
<td>1.10</td>
</tr>
<tr>
<td><em>K.pneumoniae</em></td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td><em>S.aureus</em></td>
<td>0.80</td>
<td>0.95</td>
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</table>

The range of MIC varied between 0.70 and 0.95 mg/ml against all the bacterial strains used in this study and MBC ranges between 0.85 and 1.10 mg/ml against all the bacterial clinical strains. Ascidians are already reported for rich nitrogenous source with a wide range of biological activities (Biard *et al.*, 1994).

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**REFERENCES**


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