

Antimicrobial Activity of Crude Extracts of Some Ascidiaceae (Urochordata: Ascidiaceae), from Palk Strait, (Southeast Coast of India)

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Abstract: Ascidiaceae have some pronounced pharmacological activities or other properties which are useful in the biomedical area. In the present study methanol, ethanol, hexane and butanol crude extracts from six species of ascidiaceae; (simple ascidiaceae, (*Microcosmus helleri*, *Microcosmus curvus* and *Herdmania Pallida*) and colonial ascidiaceae (*Polyclinum madrasensis*, *Didemnum psammatoide* and *Didemnum moseleyi*) were tested against eight bacterial pathogens and five fungal pathogens. In bacterial activity the maximum inhibition zone (21mm) was observed from *M. curvus* methanol extract against (*Shigella boydii*). In antifungal activity the maximum inhibition zone (9mm) was noted from methanol extract against *Aspergillus flavus*. The following orders of the maximum antimicrobial activity were observed species in level: *M. curvus* > *M. helleri* > *D. moseleyi* > *H. Pallida* > *P. madrasensis* > *Didemnum psammatoide*. The maximum zone inhibition crude extract level methanol > ethanol > hexane > butanol respectively. In conclusion, the present study was the first to analyze the antimicrobial activity from the tissue extracts of six species of ascidiaceae tested against different pathogenic bacterial and fungal strains.

Key words: Ascidiaceae % Antibacterial % Antifungal % Mandapam coast

INTRODUCTION

Ascidiaceae are sessile marine invertebrate filter feeders in the phylum Chordata that are common in benthic marine environments [1]. The number of natural products isolated from marine organisms increases rapidly and now exceeds with hundreds of new compounds being discovered every year [2, 3]. A large proportion of these natural compounds have been extracted from marine invertebrates, especially sponges, ascidiaceae, bryozoans and molluscs and some of them are currently used in clinical trials [4]. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates(ascidiaceae), sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and marine organisms [5, 6]. The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites [7]. The role of secondary metabolites as a chemical defence against epibiosis has been discussed [8 - 10]. Many class of bioactive compounds exhibiting antitumor, antileukemia, antibacterial and antiviral activity have reported worldwide [11]. The tunicate(ascidiaceae), *Trididemnum solidum* was the first marine compound to enter human cancer clinical trial as a purified natural product [12],

but was unsuccessful in further trials [13]. Nevertheless, this class of cyclic peptides provides important structural lead for a variety of antiviral, anticancer and immunosuppressant activities [14]. The potential of ascidiaceae as a source of biologically active products is largely unexplored. Hence a broad based screening of ascidiaceae for bioactive compound is necessary.

MATERIALS AND METHODS

Totally six species of ascidiaceae (*Microcosmus helleri*, *Microcosmus curvus*, *Herdmania Pallida*, *Polyclinum madrasensis*, *Didemnum psammatoide* and *Didemnum moseleyi*) were collected from Palk Strait region (Lat. 9° 17' 05.21°N, Long. 79° 10' 41.21°E) Southeast coast of India. Species were collected during low tide by while SCUBA divers. Samples were kept in ice and brought as soon as possible to the laboratory. Extraction of bioactive compound from the tissue sample was done with water, ethanol, hexane, methanol and butanol. To 5g tissues sample 5ml of water and solvents were added and ground well with mortar and pestle. Centrifugation were made at 5000 rpm for 15m to collect the supernatant to be stored in refrigerator (-20°C) until use.

Microbial Strains Used: Antibacterial activity of tissue extract was determined against ten different bacterial pathogenic, viz., *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella desentry*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Shigella boydii*, *Pseudomonas aeruginosa* and *Escherichia coli* and fungal strains viz., *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Cryptococcus neoformans* and *Mucor* sp. These clinical strains were obtained from the Department of Medical Microbiology (Raja Muthiah Medical College Hospital), Annamalai University, Annamalai Nagar.

Anti Microbial Susceptibility Assay: The antibacterial and antifungal were investigated using the standard techniques [15]. They were expressed in terms of diameter clear zone of inhibition measured in mm using vernier caliper or a scale.

RESULTS

Antibacterial and antifungal activities of crude extracts from six species of ascidians screened against 8 human pathogenic bacterial strains and 5 fungal pathogens are presented in Fig. 1- 6. Among these extracts, ethanol and methanol showed more activity against all pathogens than hexane and butanol extracts.

Effect of extract from *M. curvus* on pathogenic bacteria revealed that, the highest activity (21mm) was observed against *S. boydii* with methanol extract. Regarding ethanol extracts maximum activity (11mm) was found against *E. coli*. Hexane and butanol extract maximum activity (7mm) was found against *S. boydii*. The lowest activity (trace) was found with butanol and hexane extracts against *S. aureus* and *E. coli*. *V. cholerae*, *S. boydii*, *E. coli* and *K.pneumoniae* were highly resistant to most of the extract.

For fungi, the highest activity (8mm) was observed against *A. niger* with extract of methanol. The lowest activity (trace) was found with ethanol extract against *C. albicans* (Fig. 1).

Antibacterial activity of *M. helleri* revealed that methanol extracts showed the highest activity (19 mm), against *S. boydii*, ethanol extract revealed maximum activity (14 mm) against *E. coli*, hexane extracts showed maximum activity (8 mm) against *S. boydii* and butanol extracts revealed maximum activity (7 mm) against in

S. boydii. The trace activity was recorded for hexane and butanol extracts against *S. aureus* and *E. coli*, respectively. For fungi, the highest activity (9 mm) were recorded against *A. flavus* with extract from methanol. The lowest activity (trace) was found with ethanol extract against *C. albicans* with no activity of hexane and butanol extracts (Fig. 2).

Antibacterial activity of *H. Pallida* on pathogenic bacteria revealed the highest activity (14 mm) for methanol extract against *S. boydii*, ethanol extract maximum activity (9 mm) was found against *S. boydii* and hexane extract maximum activity (5 mm) was observed against *S. boydii*. The lowest activity (trace) was found with butanol and hexane extracts against *S. aureus*, *S.paratyphi* and *E. coli*. Hexane, ethanol, methanol and butanol extracts were not effective against *S. desentry* and *K. pneumoniae*. For fungi, the highest anti fungal activity (5 mm) highest were recorded in *A. flavus* with extract from methanol and the lowest activity (trace) was found with ethanol and methanol extract against *C. albicans* and *C. neoformans*. No activity of hexane and butanol extracts (Fig. 3).

Antibacterial activity of *P. madrasensis* revealed that, methanol extracts showed highest activity (12 mm) against *S. boydii*, ethanol extract maximum activity (7 mm) was found against *S. boydii*. The minimum activity (trace) hexane, ethanol and butanol against *S. aureus*, *S. aeruginosa* and *E. coli*, respectively and water, hexane, ethanol, methanol and butanol extracts were not effective against *V. cholerae*. for fungi the highest activity (6 mm) were recorded in *A. niger* with extract from methanol and the lowest activity (2 mm) was noted with ethanol extract against *A. niger*. No inhibition zone hexane and butanol extracts (Fig. 4).

Antibacterial activity of *D. psammatoide* revealed that, methanol extracts showed the highest activity against *E. coli* (11 mm), ethanol extract maximum activity was found against *V. cholerae* (7 mm). The minimum activity (trace) was only recorded for hexane extract against *S. boydii* and *S. aeruginosa* and water, hexane, ethanol, methanol and butanol extracts were not effective against *S. desentry*. In the hexane, ethanol, methanol and butanol extracts, no activity was found against all the species of tested fungal pathogens (Fig. 5).

Antibacterial activity of *D. moseleyi* revealed that, methanol extracts showed the highest activity (16 mm) against *S. boydii*; ethanol extract maximum activity (8 mm) was found against *S. boydii*. The minimum activity (trace)

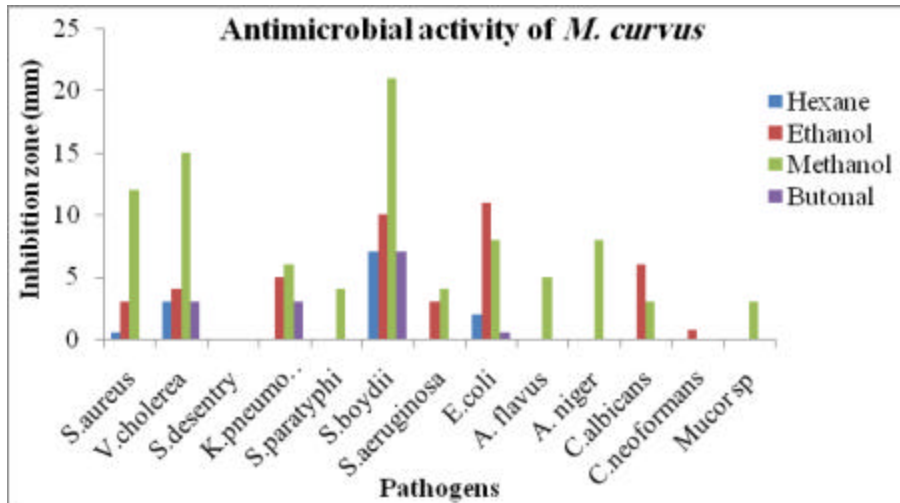


Fig. 1: Antimicrobial activity of *M. curvus*

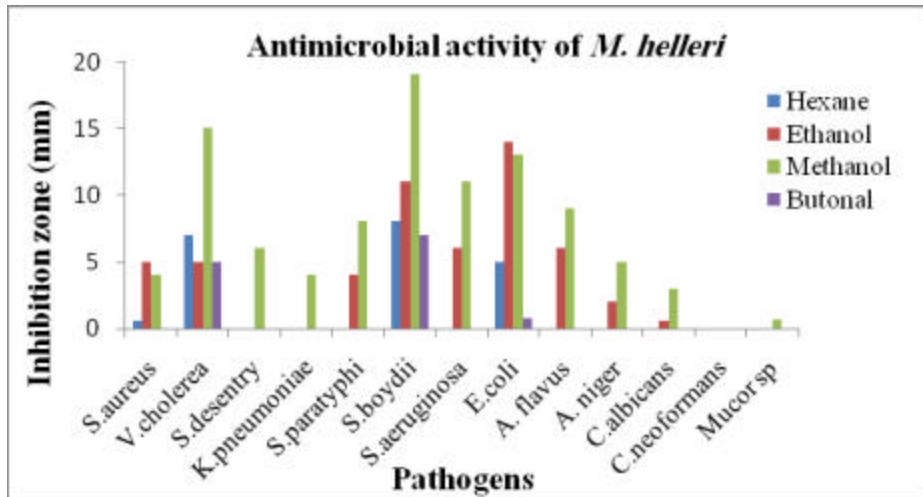


Fig. 2: Antimicrobial activity of *M. helleri*

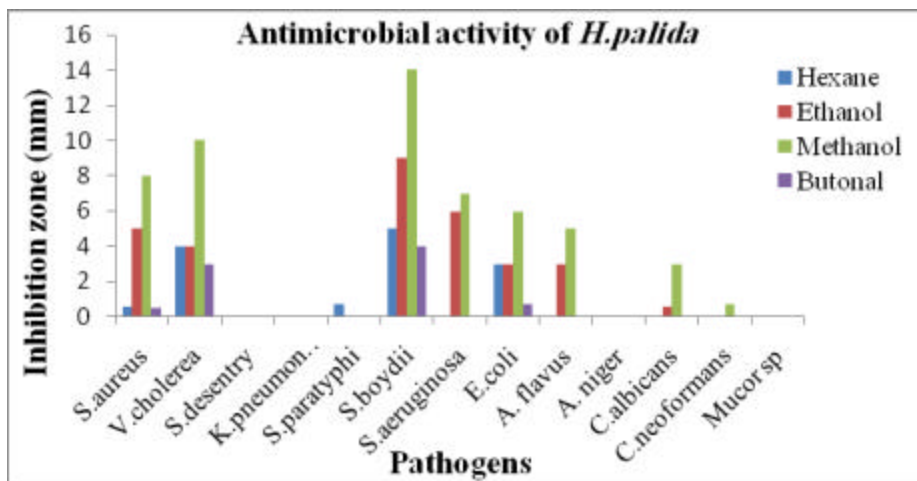


Fig. 3: Antimicrobial activity of *H. Pallida*

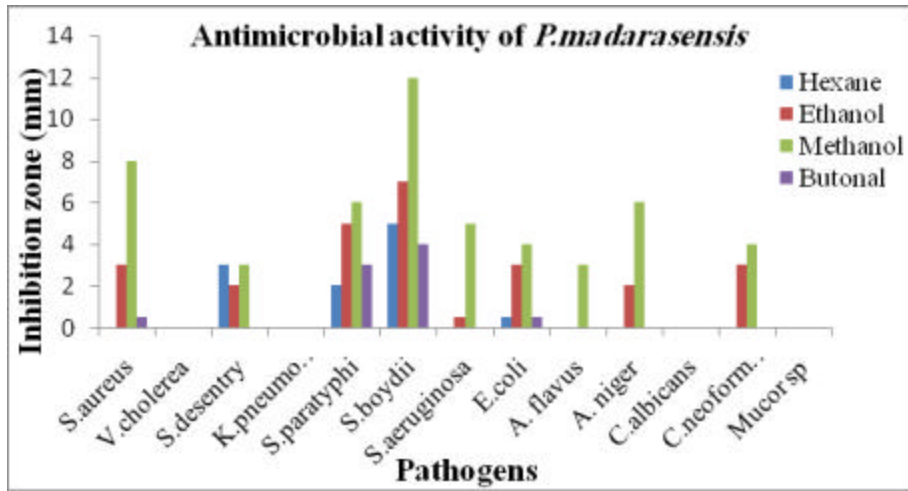


Fig. 4: Antimicrobial activity of *P. madrasensis*

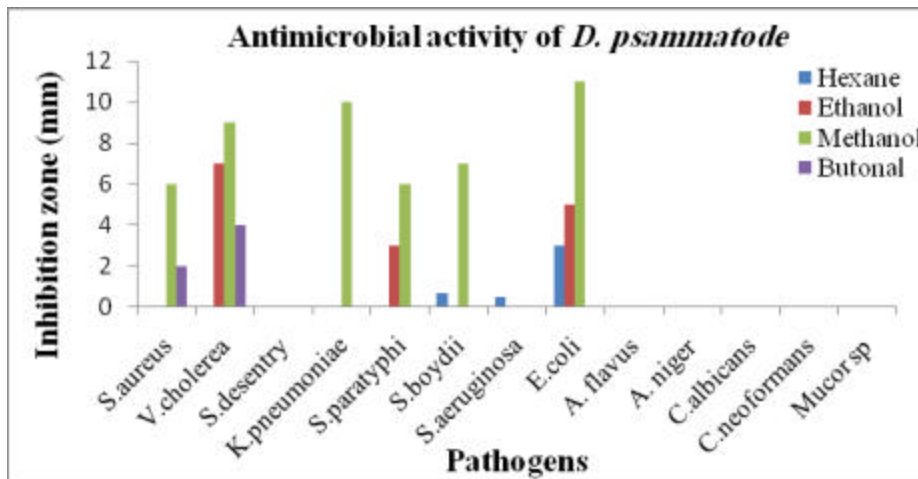


Fig. 5: Antimicrobial activity of *D. psammatoide*

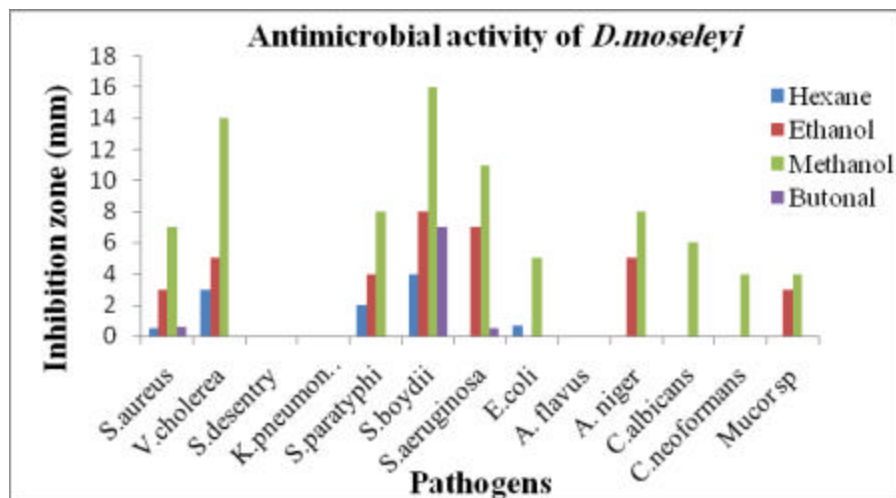


Fig. 6: Antimicrobial activity of *D. moseleyi*

hexane and butanol against *S. aureus*, *S. aeruginosa* and *E. coli*, respectively and hexane, ethanol, methanol and butanol extracts were not effective against *S. desentry* and *K. pneumoniae* for fungi, the highest activity (8 mm) were recorded in *A. niger* with extract from methanol and the lowest activity (2 mm) was found with ethanol extract against *A. niger*. Hexane and butanol extracts, no activity was found against all the species of tested fungal pathogens (Fig. 6).

DISCUSSION

In the present investigation a pronounced antimicrobial activity has been monitored against some bacterial and fungal strains. The maximum activity was recorded in methanol extract against both bacterial and fungi. Methanol extracts of *M. curvus* showed the highest activity against *S. boydii* and *V. cholerea*, ethanol extracts showed the highest activity against *E. coli* and *S. boydii*, hexane and butanol extracts showed highest activity against *S. boydii* and highest activity for fungal strains was observed against *A. niger* with extract of methanol. *M. helleri*, the highest activity was recorded for methanol extracts against *S. boydii*, ethanol extracts showed highest activity against *E. coli* and other extract showed lowest activity against *S. aureus*, *P. aeruginosa* and *S. desentry*. *H. pallida*, the highest activity was noted from methanol and ethanol extracts of against *S. boydii* and other extract showed lowest activity against *V. cholerea*, *S. paratyphi* and *P. aeruginosa* [5].

For *P. madrasensis*, the maximum activity was noted from methanol and ethanol extracts of against *S. boydii* and the lowest activity showed butanol extract against *S. desentry*, *D. psammatoide*, the maximum zone was noted from methanol extracts of against *E. coli* and butanol extract showed lowest activity against *S. aureus*. *D. moseleyi*, the maximum zone inhibition was noted from methanol and ethanol extracts of against *S. boydii* and the butanol extract showed minimum zone inhibition against *V. cholerea* and *E. coli*. Similar study antimicrobial activity in ascidians, were also made [16-19]. Whereas no anti fungal activity was observed in rest of the all fungal pathogens against hexane and butanol extract. In this respect, it was reported that the fungi are more resistant than the bacterial strains to the tested compound [20]. This could be attributed to the nature of fungal cell wall which is made up of chitin, kije the hard cover of the exoskeletons of the arthropods, which is relatively resistant, including microbial decomposition.

The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950's [21]. Since this time large numbers of marine organisms from a wide range of phyla have been screened for antimicrobial activity [22]. Many of these organisms have been antimicrobial properties, although most of the antibacterial agents that have been isolated from the marine source have not been active enough to complete with classical antimicrobial obtained from microorganisms [23].

In conclusion, Water, ethanol, methanol, hexane and butanol extracts of ascidians used in the present study showed significant antimicrobial activity compare with other solvents extraction. It is worthy to note that the product from nature source is good for health and devoid of side effects. However, further investigations involving application of the extracts as drug for human administration need more research.

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