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# Antibacterial Activity of the Shell Extracts of Marine Mollusc *Donax faba* against Pathogens

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**Abstract:** The aim of the present study is to evaluate the antibacterial activity of the shell extracts of *Donax faba* against human and fish pathogens. The shell powder of *Donax faba* were immersed separately in ethanol, acetone, methanol and cold steeped for overnight at -18°C. The antibacterial effects of the extracts were tested against six human pathogens and five fish pathogens. The extract obtained from ethanol showed a higher degree of inhibition to the human and fish pathogens. Maximum zone of inhibition was exhibited against *S. paratyphi* and *Shigella* sp. (7 mm) by the ethanol extract. The wide spectrum antibacterial activity exhibited by the shell powder extracts of *Donax faba* indicates that it may possess biologically active metabolites.

**Key words:** *Donax faba* · Antibacterial Activity · Human Pathogens · Fish Pathogens

## INTRODUCTION

The marine life constitutes almost 80% of the world biota [1] and is the sources of unique natural products used as food, fragrances, pigments, insecticides, medicines etc. More or less 10,000 pharmacologically bioactive compounds have been derived from marine invertebrates such as tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and other marine organisms [2]. The secondary metabolites derived from number of marine animals possess antibiotic, anti-parasitic, antiviral and anti-cancer activities [3,4]. The Tyrian purple an ancient dye pigment is the first natural product of marine origin reported in literatures

Among the marine invertebrates, the molluscs are the potential source of bioactive substances. The bioactive compounds isolated from the gastropods are considered to have a role in the chemical defense of the animals against their predators. Molluscs in the oceans are common sight and are virtually untapped resource for the discovery of novel compounds. Many studies on bioactive compounds from molluscs exhibiting antitumour, antileukaemic, antibacterial and antiviral activities have been reported worldwide from *Phyllidae* sp. [5], bivalves [6] and gastropods [7]. Bioactive metabolites from molluscs such as sea hare [8], *Chromodoris* sp [9], *Onhidella* sp [10] were isolated and structurally elucidated. Currently, there is an increasing interest on the bioactivity of molluscan extracts and secondary metabolites eventhough the overall secondary metabolites investigated from molluscan species form a tiny proportion (<1%). Some marine gastropods and bivalves have been of great interest to natural products chemists, yielding a diversity of chemical classes and several drug leads currently in clinical trials. Many molluscs mantle cavity produces mucus e.g. Muricid gastropods (rock snail) which defend the developing larvae against microbial infection [11].In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[12]. Moreover, cost of production of synthetic drugs is also high and they cause adverse effect when compared to bioactive naturally derived drugs [4]. Hence, intense research is under progress towards the search for natural remedies with potent biological activities from marine organisms [13].

Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemic, antibacterialand antiviral activities have been reported earlier [14-17]. Considering the importance of the marine natural products, in the present study, an attempt was made to investigate the antimicrobial activity of the shell extracts of *Donax faba*.

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## MATERIALS AND METHODS

**Preparation of *Donax faba* Powder:** Live bivalve *Donax faba* were collected from the inter tidel area of Tuticorin coast. The shells were broken and the soft tissues were removed and the shells were washed thoroughly with distilled water and then immersed in fresh lime juice for 4 hours for purification. Then the shells were washed with distilled water, air dried and the shell powder was prepared according to the method of Narayanasamy [18].

**Preparation of Shell Extracts:** Approximately 5g of shell powder was immersed separately into ethanol, methanol and acetone solvents and they were cold steeped at -18°C. The extracts from each solvent were filtered twice using Whatman No.1 filter paper. Samples were centrifuged at 5000 rpm for 15 min. and the supernatant were collected. Then it was used for the experimental work.

**Microbial Cultures:** Five species of human pathogens; *Bacillus cereus, Proteus vulgaris, Shigellady senteriae, Salmonella paratyphi, Shigella* sp and *Streptococcus mutants* were obtained from the Christian Medical College Hospital, Vellore. Five fish pathogens; *Vibrio harveyi, Vibrio sclintis, Vibrio parahaemolyticus, Vibrio alginolyticus* and *Vibrio anguillarum* were obtained from Fisheries Department, Cochin.

**Inoculum Preparation for Bacterial Strains:** Standard Microbial techniques were followed for media preparation and other routine process. Nutrient broth (Himedia) was prepared and sterilized in an autoclave at 121°C, 15 lbs pressure for 15 min. The bacterial strains were individually inoculated in sterilized nutrient broth and were incubated at 37°C for 24 hoursand and used in the test proper.

**Antibacterial Activity:** Antibacterial activities of the extracts were analyzed using well diffusion technique followed by Ramasamy [19]. Twenty four hours old nutrient broth cultures of test bacteria was aseptically swabbed on sterile Mueller Hinton agar plates. The wells with 5mm diameter were punched with a sterile cork borer on to the Muller Hinton agar plates. The wells were filled with 50 µL of the extract. The wells containing the solvent alone were used as negative control. Plates were incubated at 37°C for 24 hours. Antibacterial activities were evaluated by measuring the zone of inhibition showed in millimetres.

## RESULTS

**Antibacterial Activity:** Three extracts of *Donax faba* were screened for antibacterial activity against six human and five fish pathogens were represented in the Fig. 1 and 2. From the three solvent extracts of *Donax faba,* the ethanol extract were able to inhibit all the human pathogens exhibiting broad spectral antibiotic activity. Ethanol extract from *Donax faba* showed highest activity against *Salmonella paratyphi* and *Shigella* sp. (7mm) and the lowest activity was found with acetone extract against *Bacillus cereus* and *Salmonella paratyphi* (4mm). Similarly the methanol extract of *Donax faba* does not showed any activity against the six human pathogens (Table 1). The ethanol extract showed maximum activity against the fish pathogen of *Vibrio parahaemolyticus* (5mm) and minimum activity against *Vibrio harveyi* (3mm). The acetone extract showed maximum activity against *Vibrio parahameolyticus* (6mm). Methonal extract did not show any activity against all the fish pathogens (Table 2).

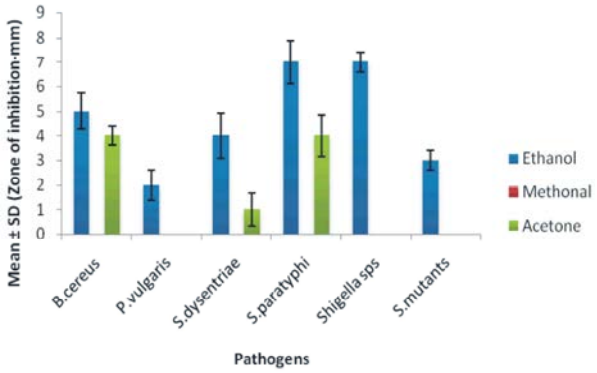


Fig. 1: Antibacterial activity of shell extracts of *Donax faba* against human pathogens

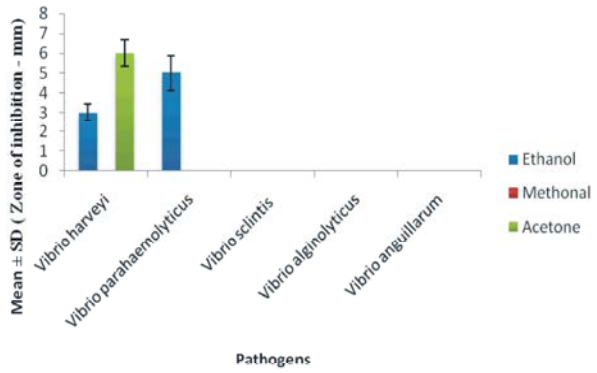


Fig. 2: Antibacterial activity of shell extracts of *Donax faba* against fish pathogens

Table 1: Activity of shell extracts of *Donax faba* against human pathogens

| Pathogens | Solvents | | |
|---|---|---|---|
| | Ethanol | Methanol | Acetone |
| | Zone of inhibition (mm) | | |
| B.cereus | 5± 0.75 | - | 4±0.37 |
| *P.vulgaris* | 2± 0.62 | - | - |
| *S.dysentriae* | 4± 0.93 | - | 1±0.680 |
| *S.paratyphi* | 7± 0.88 | - | 4±0.85 |
| *Shigella* sp. | 7± 0.42 | - | - |
| *S.mutants* | 3± 0.39 | - | - |

Table 2: Antibacterial activity of shell extracts of *Donax faba* Against fFish pathogens

| Pathogens | Solvents | | |
|---|---|---|---|
| | Ethanol | Methanol | Acetone |
| | Zone of inhibition (mm) | | |
| *Vibrio harveyi* | 3±0.45 | - | 6±0.67 |
| *Vibrio parahaemolyticus* | 5±0.88 | - | - |
| *Vibrio sclintis* | - | - | - |
| *Vibrio alginolyticus* | - | - | - |
| *Vibrio anguillarum* | - | - | - |

## DISCUSSION

In recent years, great attention has been paid to study the bioactivity of natural products and their potential pharmacological utilization. Among the marine invertebrates, the molluscs are the potential source of bioactive substances. The bioactive compounds isolated from the molluscs, in particular from the gastropods are considered to have a role in the chemical defence of the animals against their predators. Many promising lead compounds have been reported from marine mollusc having anti-inflammatory activity [9]. Nevertheless, in most cases, there has been no scientific research undertaken to substantiate the health benefits derived from molluscs and the active ingredients in the taxa involved are typically unknown. The first attempt to locate antimicrobial activity in marine organisms was initiated around 1950s [20]. Since this time, a large number of marine organisms from a wide range of phyla have been screened for antimicrobial activity [21]. Many of these organisms have been antimicrobial properties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to compete with classical antimicrobial obtained from microorganisms [22]. In the present study, Ethanol extract from *Donax faba* showed the highest activity against *Salmonellaparatyphi* and *Shigella* sp.(7mm) the lowest activity was found with acetone extract against *Bacillus cereus* and *Salmonella paratyphi* (4mm). Similarly the methanol extract of *Donax faba* did not show any activity against the six human pathogens. The ethanol extract showed maximum activity against fish pathogen *Vibrio parahaemolyticus*(5mm) and minimum activity against *Vibrio harveyi* (3mm), acetone extract showed maximum activity against *Vibrio parahameolyticus* (6mm). Methonal extract did not show any activity against all fish pathogens. Grasian Immanuel [23] prepared solvent extracts of the shell powder of *C. moneta* solvent extract and investigated the antibacterial effect against three opportunistic human pathogens such as *P. vulgaris*, *Micrococcus* sp. and *S. abory* and found that the growth of all the three pathogens was inhibited.

## CONCLUSION

The shell extracts of *Donax faba* possess bioactive compounds that have potent antibacterial effect. Further studies are essential to explore those bioactive compounds and to convert them into usable drugs.

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