Screening and Characterization of Antibacterial Compounds from Some Marine Sponge Species

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Abstract:
Objective: The antibacterial activities were done by using of various solvents such as ethanol, petroleum ether, methanol, chloroform, n-butanol and ethyl acetate extracts of various marine sponges like *Callyspongia reticutis* Dendy (*C. reticutis*), *Thalysias vulpine* Lamark (*T. vulpine*), *Echinodictyum gorgonoides* Dendy (*E. gorgonoides*) and *Callyspongia diffusa* Ridley (*C. diffusa*), *Gelliodes cellaria* Rao (*G. cellaria*) against gram positive and gram negative bacterial pathogens like *Pseudomonas aeruginosa* PA01 and *Proteus mirabilis* ATCC 7002 respectively. **Methods:** The nutrient agar well diffusion method is used to find the antibacterialbial activities against various sponge extracts. The functional groups of the sponge extracts are specifically mitigate bacterial metabolic intermediates determined by FTIR. **Results:** The antibacterial activities of the sponge crude extracts were increased with different concentrations in the agar well plates to indicate the presence zone of clearance. The crude extracts were prepared from different solvents such as the n-butanol and chloroform extract was the most effective extracts. At this stage the gram negative bacteria *Proteus mirabilis* (*P. mirabilis*) and the gram positive bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) appear to be most sensitive strain while and *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia*. The *Staphylococcus aureus* indicate resistance to the various tested concentrations and have response to no zone of inhibition was observed. The inhibition of microbial growth at concentration as low as ~50 -150 mg/mL indicated the potent antibacterial activity of above mentioned porifera sponge extracts. **Conclusions:** These research works results were find critically industrially important compounds from selected sponge extracts and the functional groups of plant compounds is responsible for great antibacterial activity.

Keywords: Pathogens, Antibacterial, Drugs,, Solvents, *Callyspongia, Gelliodes, Echinodictyum*, compounds, *Proteus*, *Pseudomonas*, remove, functional groups

1. Introduction

Microorganisms are called as universal organism, found in everywhere; some are pathogenic or beneficial in nature. Due to the human activities increases the pollution in environment helps to emergence of many pathogens like bacteria, viruses and other unicellular organisms that causes serious diseases to humans.

The living organisms include viruses, archaea, eubacteria, microscopic fungi, protozoa and unicellular algae. Among multimillions of living things, the eubacteria are predominantly causes several infections or diseases in higher plants and animals. Microorganisms such as bacteria are ubiquitous in everywhere and have the ability to do various beneficial and harmful functions. The beneficial functions of bacteria include

environmental protection by nitrogen fixation and environmental scavenging mechanisms. Some microorganisms do digestive functions in humans. But most of the bacteria cause severe deadly diseases to humans, animals and plants.

In worldwide population [1] More than 15 millions of death observed due to bacterial infections. The bacterial diseases responsible for serious health problems to people and leading to high mortality around the earth [2] Now a days, medical drugs have very low activity and cause many side effects but the in order to find the better phytochemical compounds from natural sources is very important. Recently, the viral, fungal or bacterial drug resistance is leads to increase the opportunistic infections to humans [3]. The Gram positive bacteria cause severe diseases than Gram negative bacteria, but some Gram negative bacteria also causes diseases to both organisms. *Pseudomonas aeruginosa* causes various diseases like, endocarditis, pneumonia, endophthalmitis, meningitis, septicemia and malignant external otitis. Many pathogenic bacteria cause severely diseases to plants, animals and human beings.

The *Proteus mirabilis* is one of the gram negative pathogenic bacteria and causes dedly diseases to animals, plants and humans. It causes burn infections, wound infections. cystitis, Urinary Tract Infections and prostatisitis rarely causes otitis media, meningo-encephalitis, chronic suppurative meningitis, eye infections (endophthalmitis) and respiratory tract infections [4]. Some other species cases has been reported to causes empyema, endocarditits, mastoiditis and cellulitis osteomyelitis. It also was causes rheumatoid arthritis.

The global environment consists of 29% land with 71% water in the global level and has many varieties of biodiversity observed in both environments. In marine environment have larger biodiversity than our terrestrial environment. The marine diversity has more numbers of organisms such as micro & macroalgae, corals, mangroves, zooplanktons, marine animals and sponges. Among the bioresources, the sponges having industrially important and belongs to phylum porifera [5-6].

Most sponges have potent pharmaceutical compounds especially alkaloids, terpenoids, phenolics and other secondary metabolites [7] The marine sponges like *Callyspongia reticutis* Dendy (*C. reticutis*) belong to the family of Callyspongiidae, *Thalysias vulpine* Lamark (*T. vulpine*) have the family is Lophomnidae, Phorbasidae is the family of *Echinodictyum gorgonoides* Dendy (*E. gorgonoides*) and *Callyspongia diffusa* Ridley (*C. diffusa*) belong to the family of Callyspongiidae and *Gelliodes cellaria* Rao (*G. cellaria*) have the family of Niphatidae.

Most sponges are generally grow in more salt place environment is called halophiles in the class of calcaria Most marine organisms have very much phytochemical properties than terrestrial organisms products [8]. The antifungal and antimicrobial compounds from marine sponges are very excellent natural remedy to remove the communicable and non-communicable diseases of humans and animals [5 & 9-10]. Now a day's the pathogens are very epidemic to all organisms including plants, animals and humans.

The alternative source used to develop novel drugs from sponge secondary metabolites. Previously these marine sponges antibacterial activity were not investigated elaborately, in this study to find the antibacterial activity of selected bacterial pathogens using selected marine sponges against various solvents like Petroleum ether, methyl acetate, ethanol, methanol, Chloroform and n- Butanol Finally Fourier Transform Infrared Spectroscopy technique employ to find the sponge secondary metabolites is responsible to interact or remove the bacterial metabolites, proteins and nucleic acids in the antibacterial activity.

2. Materials and Methods

2.1. Plant collection

Callyspongia reticutis, Thalysias vulpine, *Echinodictyum gorgonoides, Callyspongia diffusa* and *Gelliodes cellaria* were collected from Thondi region of Rameswaram District, Tamil Nadu. The taxonomical identification of this sponges

was done by Marine Sponges of Tamil Nadu Publication from M.S. Swaminathan Research Foundation.

2.2. Sponge preparation and extraction

The fresh sponges was washed well under running tap water and dried in a warm for 3 to 5 days. The samples were grinded into fine powder and extract prepared by different solvents such as ethanol, methanol, petroleum ether, chloroform, n-butanol and ethyl acetate.

2.3. *Samples preparation*

The extract preparation by adding 0.5g sample powder in 10 ml solvents and kept into shaker then collected solvent layer were dried in water bath. Finally the dried samples were kept into 2ml eppendorf to store for future use.

2.4. *Bacterial strains*

Target pathogens used in this study was *Proteus mirabilis ATCC 7002* and *Pseudomonas aeruginosa PA01*. Both the cultures were streaked and maintained in Luria – Bertan agar plates. For antibacterial assay, both the strains were cultivated in sterile 2ml of nutrient broth for overnight and 1% inoculums was sub cultured for 3 hours in 2 ml of sterile nutrient broth.

2.5. Antibacterial activity through agar well diffusion assay

The agar well diffusion assay was performed in nutrient agar plates. The nutrient agar medium was sterilized at 121°C for 20 minutes and poured in sterile petriplates. Plates were allowed to solidify. Then the sub-cultured test pathogens were swabbed on the nutrient agar plates and kept for few minutes for drying. Wells with 5mm diameter was made in each plate and 100µl of crude extract obtained from sponges were loaded on the well. The plates were incubated for 16 hours at 30°C and the zone of inhibition against each pathogen was measured.

2.5. FTIR analysis of crude of sponges

The active compounds from sponge samples with various solvents were examined under FTIR Spectroscopy. FTIR spectrum of crude extract mixed with potassium bromide pellet was recorded using FTIR spectrophotometer.

3. Results

3.1 Weight of crude extract obtained through various solvent extractions:

The shade dried samples of sponges were extracted with various solvents and the weight of the each crude extract was weighed in a pre weighed eppendorf tube. The results were given in Figure 1 & 2 and Plate 1.

3.2 Antibacterial activity of n-butanol extract of sponges:

A total of n-Butanol extract of five different sponges were tested for their antibacterial activity against target pathogens. Out of which, the crude extract of S1, S3 and S5 showed higher inhibitory zones like 30mm, 18mm and 22mm respectively against *P. mirabilis* (Fig.1 & 2 and Plates 1).

3.3 Antibacterial activity of chloroform extract of sponges:

Sponge powder soaked in chloroform extracts were tested for their antibacterial activity against target pathogens. Out of which, the crude extract of S1 and S4 showed an inhibitory zone of 20 mm and 17 mm respectively against *P. mirabilis* (Fig.1 and 2).

3.4 Antibacterial activity of Ethanol extract of sponges:

The ethanol extract were prepared with different sponge samples tested for their antibacterial activity against target pathogens, the crude extracts like S2 and S5 showed an inhibitory zone of 13mm and 11mm respectively against *P. mirabilis* (Fig.1 and 2).

3.2 Antibacterial activity of n-butanol extract of sponges:

A total of n-Butanol extract of five different sponges were tested for their antibacterial activity against target pathogens. Out of which, the crude extract of S2 and S5 showed higher inhibitory zones like 13mm and 11mm respectively against *P. aerugenosa* (Fig 2 and Plates 1)

4. FTIR analysis of crude extract of sponges:

The FTIR (Fourier Transform Infrared Spectroscopy) was done to identify the functional group of the antibacterial compound present in the crude extract of S1. The FTIR peaks of crude extract of S1 showed the presence of amide, phenol, nitro and alkane groups (Fig.3).

Plates 1. The Maximum Antibacterial activity in terms of zone of inhibition of different marine sponge extracts like S1 in n-Butanol and S1 & S4 in chloroform against *Proteus mirabilis*. The zone of inhibition also observed the extracts of marine sponge extracts like S3 in n-Butanol against *Pseudomonas aeruginosa*.

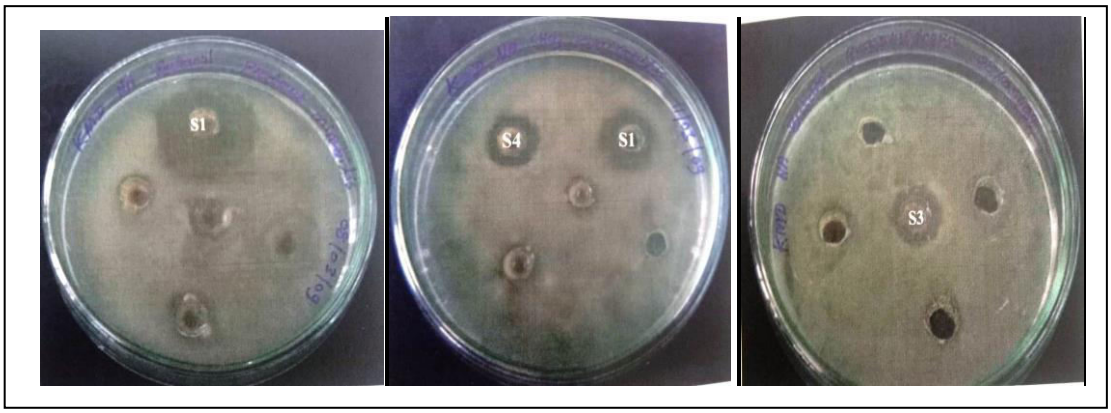
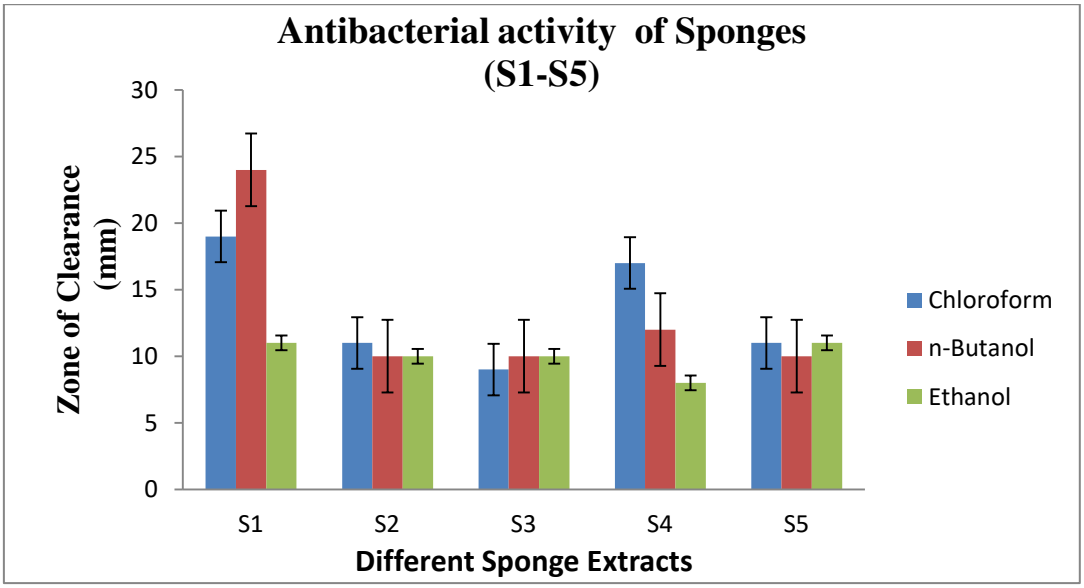
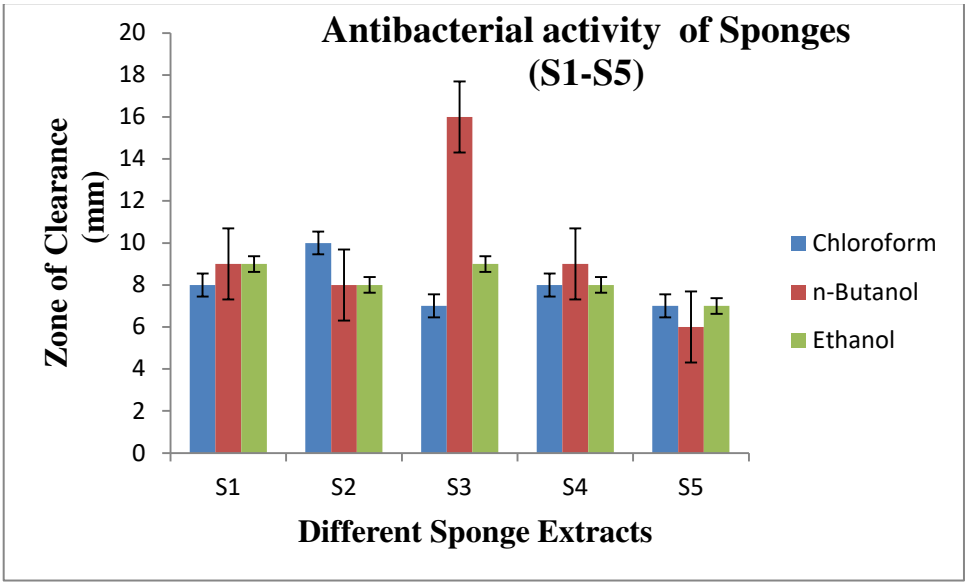


Figure 1. Antibacterial activity against *Proteus mirabilis* in terms of zone of inhibition of different sponge extracts (S1 to S5) in different solvent systems



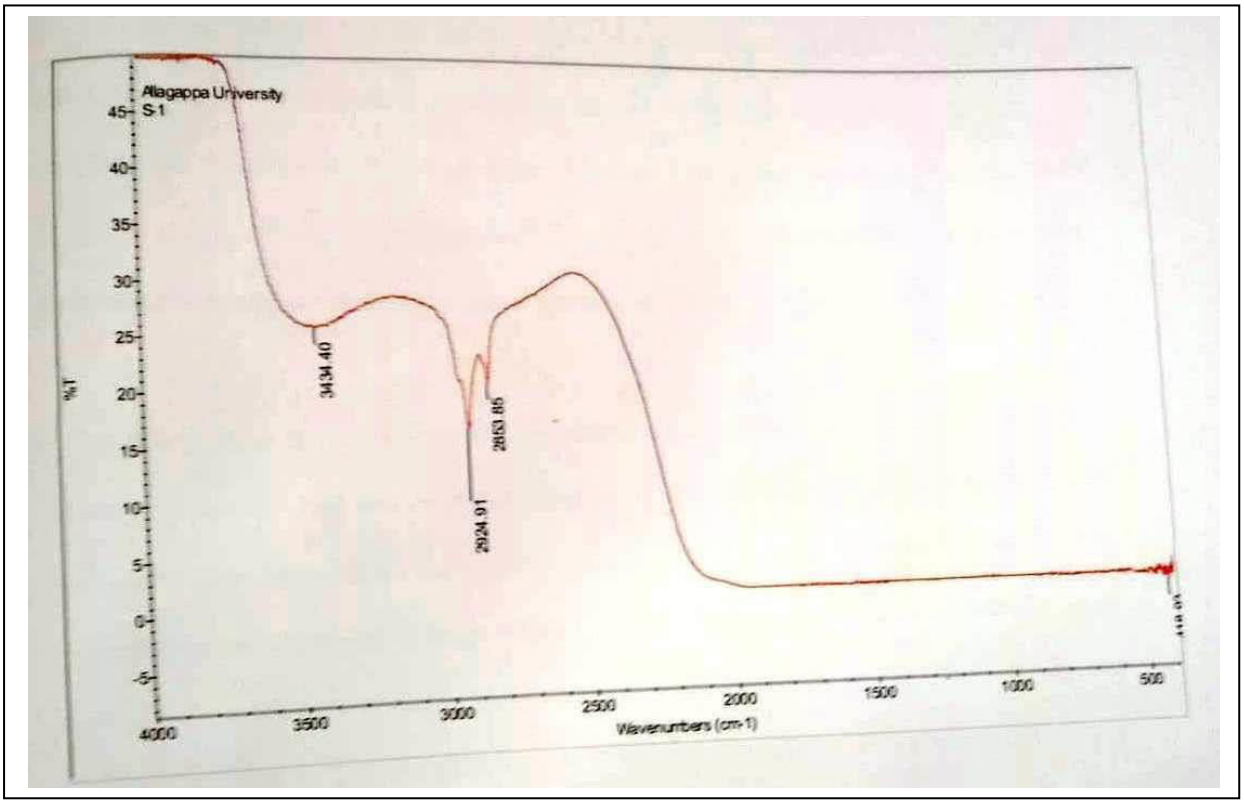
Note: S1 = *Callyspongia reticutis* S2 = *Thalysias vulpine* S3 = *Echinodictyum gorgonoides* S4 = *Callyspongia diffusa* S5 = *Gelliodes cellaria*

Figure 2. Antibacterial activity against *Pseudomonas aeruginosa* in terms of zone of inhibition of different sponge extracts (S1 to S5) in different solvent systems



Note: S1 = *Callyspongia reticutis* S2 = *Thalysias vulpine* S3 = *Echinodictyum gorgonoides* S4 = *Callyspongia diffusa* S5 = *Gelliodes cellaria*

Figure 3. FTIR spectrum of Chloroform extracts of sponge of S1



4. Discussion

Recently the sponges are the very important sources of medicine to rectify so many diseases. The sponges is belongs to animal kingdom but it is frequently adapt many disturbances of many marine predators such as turtles, fishes and other invertebrates, So the sponges produces various chemicals such as secondary metabolites to deter their predators [11].

The sponges are always sessile in nature and have huge diversity of secondary metabolites [12]. These sponge drugs have many potential properties such as antiviral, antibacterial, antihelminthic, antifungal or antimalarial activities. The secondary metabolites from the sponge called phyochemical compounds that inhibit the action against various communicable and non-communicable diseases [13]. However, the extraction of 5300 new compounds from sponges identified [14]. Betterment of the biological activities and or chemical constituents of sponges is desirable not only for the discovery of new therapeutic drugs, but because such information may be of value in disclosing the new sources of already known biologically active compounds such as alkaloids, tannins, flavonoids, and phenolics and terpenoides, these bioactive compounds shows excellent antimicrobial activity [15]. The sponges were cut into small pieces and homogenized by crushing in a mortar and pestle [16]. Similarly in our study is done by the sponges homogenized by using mortar and pestle.

There are many different solvent systems such as n-butanol, chloroform, ethanol, acetone and ether are used for plant compounds extraction. In our study also, the powdered sponges of entire body were extracted with solvents such as petroleum

ether, ethanol, methanol, butanol, ethyl acetate and chloroform [17]. Different sponge extracts were prepared using various solvents such as chloroform, ethanol, n-butanol, petroleum ether and water for the analysis of antibacterial activity analysis by standard agar well diffusion assay method [18-19]. Similarly in the present study different solvent systems like n-butanol, chloroform and ethanol extracts prepared and the antibacterial analysis by agar well diffusion in nutrient agar plates.

The crude extract of sponges had strong inhibitory action against gram negative pathogens [20]. Similarly in the present study also the n-butanol crude extract of sponges S1 and S5 showed highest antibacterial activity against *P. mirabilis* ATCC 7002 with the inhibitory zone of 24 mm and 20 mm respectively against *P. mirabilis*. In the same way the chloroform crude extract of sponges S1 showed high antibacterial activity against *P. mirabilis* ATCC 7002 with the inhibitory zone of 24 mm against *P. mirabilis*. In ethanol crude extract of sponges S1 and S5 showed medium antibacterial activity against *P. mirabilis* ATCC 7002 with the inhibitory zone of 17 mm and 14 mm respectively against *P. mirabilis* (Fig1 & Plate 1).

The antibacterial activity also observed in some solvent extracts of sponges against some gram positive pathogens [20]. Similarly in the present study also the n-butanol crude extract of sponges S3 4 showed highest antibacterial activity against *P. aeruginosa* PA01 with the inhibitory zone of 17 mm against *P. aeruginosa* (Fig.2 & Plate 1).

The crude antibacterial compound of sponges S1 was further studied using FTIR spectrophotometer to identify the presence of functional groups present in the crude extracts. FTIR spectrum of crude extract (n-butanol) of S1 showed presence of phenol, nitro, amide and alkane groups (Fig.3). These chemical groups reveal that the presence and or antoibacterial activity of secondary metabolites such as alkaloids, terpenoides, phenolic compounds, tannins and saponins as same as reported in GCMS analysis [21-27].

5. Conclusion

The present study indicates the presence of an active antibacterial compound in marine sponges like *Callyspongia reticutis, Thalysias vulpine*, *Echinodictyum gorgonoides, Callyspongia diffusa* and *Gelliodes cellaria*. The results support that sponge *C. Reticutis, E. gorgonoides, C. diffusa* and *G. cellaria* could be a valuable source of novel substances for future drug discovery. A detailed investigation has to be done with the objective of

isolating biologically important active compounds along with the search for new novel macromolecules is currently under the study.

6. References

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