ANTIBACTERIAL ACTIVITY OF CRUDE METHANOLIC AND FRACTIONATED EXTRACTS OF *AAPTOS SUBERITOIDES*

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ABSTRACT

In this research, we at the first time assessed antibacterial effects of methanolic crude and fractionated extracts from the marine sponge, *Aaptos suberitoides* against three bacterial strains including *E. coli., S. aureus, B. cereus.* The anti-bacterial assays were carried out by the disc diffusion method to test the anti-bacterial activity of different concentrations of methanolic crude of *A. suberitoides* against the indicator bacterial pathogens. The results indicated that extracts of *A. suberitoides* exhibited anti-microbial activity against all tested bacterial strains in concentration-dependent manner. Moreover, we showed that the majority of the active compound was in the polar fractions.

Keywords: antibacterial; *Aaptos suberitoides*; *active compound; marine organisms*.

1. Introduction

Marine invertebrates such as sponges are well-known sources of a wide variety of natural products. Marine organisms evolved differently from terrestrial organisms. Because of greater competition of existence in their habitat, marine organisms secrete antibacterial proteins and bioactive secondary metabolites for bacterial defense as part of their metabolism that help them survive (Zilinskas RA, 1992). Marine sponges, being sessile, are one of the marine organisms that attract the interest of many natural product chemists because of its unique characteristics in adapting to the harsh and diverse marine environment.

Vietnam offers vast potentials from its marine resources, sponges in particular, waiting to be utilized as source of essential bioactive substances with pronounced pharmacological potentials. The challenge now lies in the effective exploration of the rich chemical diversity offered by marine life.

Natural products have long served as sources of antibacterial agents. The discovery of the antibiotic activity of *Penicillium notatum* in 1929 by Alexander Fleming has revolutionized medical science, leading to the invention of the wonder drug penicillin. Other well-known examples included cephalosporin, vancomycin, and carbapenem. However, with the increasing usage of these drugs, bacterial strains that develop drug resistance to these antibiotics have been reported everywhere (Bassetti M et al, 2013; Rice LB, 2006). Thus, the development of new drugs or alternative therapies is clearly a matter of urgency. Natural products derived from marine organisms are structurally diverse and differ from those identified from terrestrial organisms. They can be used to design and to develop news, therapeutic agents. It is clear that many marine organisms have not yet been extensively studied and can serve as a vast

source of potential ready-made pharmaceutical agents (Aneiros A et al, 2004).

The oceans occupy a large proportion of the Earth's surface and the bulk of the biosphere. Marine organisms produce a constellation of biomolecules for survival in an environment in which they face intense competition with pathogenic microbes (Ammerman J et al, 1984). Bioactive compounds from terrestrial organisms have a long history of medicinal applications when compared with the notorious marine compounds, such as shellfish paralytic toxins, fish neurotoxins, and cyanotoxin from cyanobacteria. In the recent years, there is growing attention to an alternative view of marine natural products (Chin Y-W et al, 2006; Tsoulkalas N, 2014). They provide a rich source of chemical diversity that can be used to design and develop new, potentially useful therapeutic agents (Barboza NM, 2012; Tsoukalas N, 2014). The vast majority of currently used antibiotics have been isolated from terrestrial sources, and recent researchs strongly suggests that the marine environment represents an untapped source of new bioactive molecules (Kang HK et al, 2015; Valliappan K, 2014). In this respect, marine bacteria and fungi seem to be the most prominent sources for antibacterial agent due to their diversity and ability to grow rapidly and sustainably in bioreactors. Other sources, like algae, sponges, corals, mollusks, and other marine animals, can also supply interesting scaffolds or leads for drug discovery, which can be reproduced through chemical synthesis.

This research investigate anti-bacteia effects of methanol extracts of one metabolite from the marine sponge, *Aaptos suberitoides* (Ammar MSA et al, 2007). This marine sponge is a yellow-brown sponge classified under Demospongiae, order Hadromerida, family Suberitidae and genus Aaptos. The distribution of these sponges span within the region of Vietnam, Malaysia, Indonesia, Palau, and have fairly widespread in South East Asian wasters. Report of its abundance also includes the Red Sea, Egypt (Ammar MSA, 2007). In this research, we have found that methanol extract of *A. suberitoides* showed potential antibacterial on *E. coli*, *S. aureus* and *B. cereus*.

2. Materials and Methods

Sponge collection

Aaptos suberitoides sponge was collected from the reef in the Hon Mot - Nha Trang Bay, Viet Nam. The sponge was collected at 5 to 10 m depth. The sponge sample was immediately frozen after collection and maintained at -20°C prior to extraction.

Extraction

The sponge (whole body) was allowed to thaw, cut or crushed into small pieces, shade dried, and was sent frozen and freeze-dried. The dried sponge was crushed into powder using a pestle and mortar to provide a greater surface area. The powder should be sufficient to fill the porous cellulose thimble (~15 g) which is placed inside the Soxhlet extractor containing 150 ml methanol. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 6 hours. Once the process has finished, the methanol should be evaporated using a rotary evaporator, leaving a small yield of extracted sponge material (about 2 to 3 ml) in the glass bottom flask.

Microorganisms

Staphylococcus aureus subsp. *aureus* Rosenbach (ATCC® 25923™) (ATCC 29213), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) and *Bacilus cereus* (isolated) were chosen based on their clinical and pharmacological importance. The

bacterial strains obtained from Biotechnology Center of Ho Chi Minh City, Viet Nam, were used for evaluating antimicrobial activity. The bacterial stock culture was incubated for 24 hours at 37°C on nutrient medium, following refrigeration storage at 4°C. The bacterial strains were grown in Trypton Soy Agar (TSA) plates at 37°C. The stock cultures were maintained at 4°C.

Determination of zone of inhibition method (ZDI)

For the determination of zone of inhibition, the sponge crude methanol extract and fractionated extracts were screened for their effects against the *E. coli*, *S. aureus* and *B. cereus*. The sets of three dilutions (25, 50 and 100 µg/ml) of *A. suberitoides* methanol crude extract were prepared in double-distilled water using nutrient agar tubes. TSA sterile agar plates were seeded with indicator bacterial strains (10^7 CFU/ml) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar conditions by using tetracyline (100 µg/ml) for antibacterial activity. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C. The sensitivity of the microorganism species to the sponge extracts was determined by measuring the sizes of inhibitory zones (excluding the diameter of disc) on the agar surface around the disks. The zone of inhibition of bacteria around the disc (average of three experiments) was measured, and we scored the assay as resistant if it was <5 mm, intermediately if <10 mm, and susceptible if it was >10 mm.

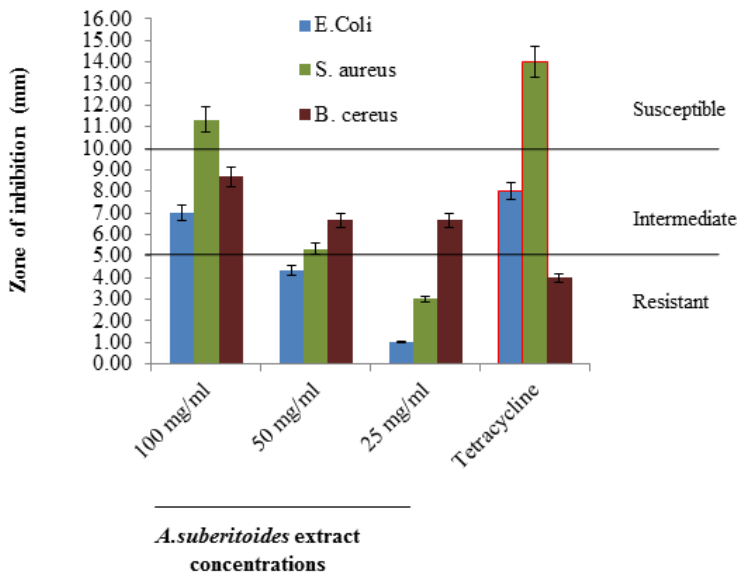
Statistical analysis

All data were expressed as the mean ± standard deviation. Statistical analyses were done using Student's *t-test*, in which $p < 0.05$ was the minimum requirement for a statistically significant difference.

3. Results and Discussion

Anti-bacterial activity of crude methanol extracts form *A. suberitoides*

The anti-bacterial assays were carried out by the disc diffusion method to test the antibacterial activity of different concentrations of methanol crude extract (25; 50 and 100 mg/ml) form *A. suberitoides* against the indicator bacterial pathogens. The results as show in Figure 1 indicated that extracts of *A. suberitoides* exhibited anti-microbial activity against all 3 tested bacterial strains. The antibacterial activities of sponge extracts were compared with inhibition zones around the commercial tetracycline antibiotic discs as references.



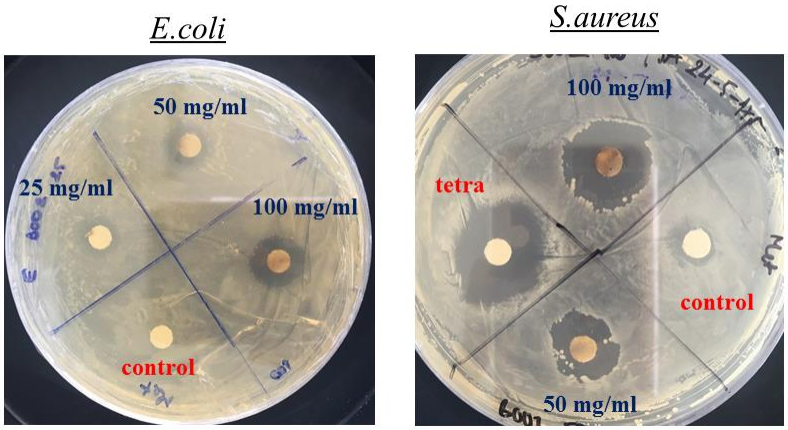


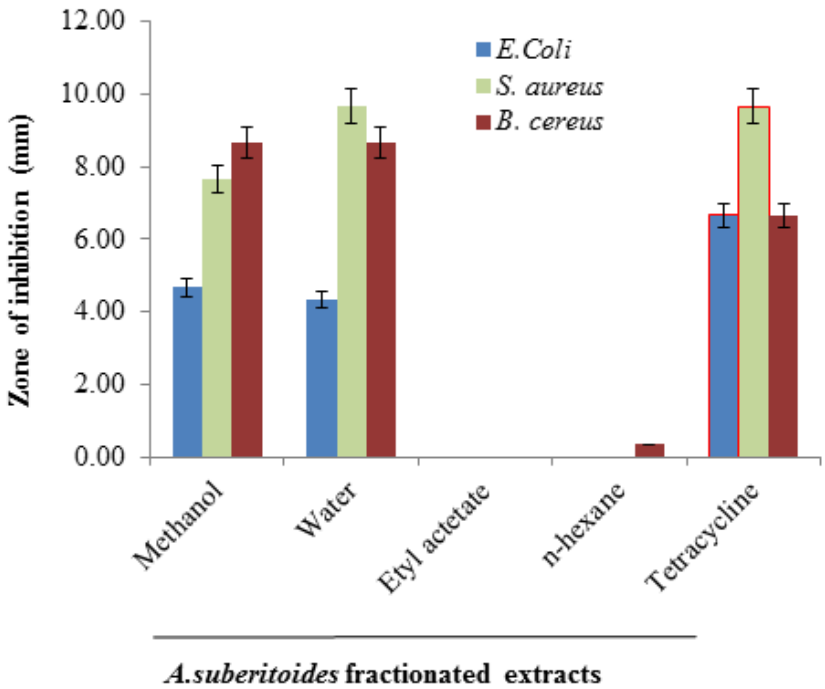
Figure 1. Anti-bacterial activity of *A. Suberitoides* methanolic crude extract against the indicator bacterial pathogens in concentration-dependent manner following agar-well diffusion method

E. coli, *S. aureus* and *B. cereus*. were treated with 25, 50 or 100 mg/ml *A. Suberitoides* methanolic crude extracts. Tetracycline was used as positive control. Data presented are the means of three replicates. Values are expressed as mean±SD of three replicates

Antibacterial activity of fractionated extracts of *A. suberitoides*

The extraction of biologically active components of the organisms was carried out with different solvents in order of increasing polarity including n-hexane, ethyl acetate, water, and methanol. The results in figure 2 indicated that the compounds were activated in the aqueous and methanol fractions and were not presented in non-polarity solvent n-hexane and moderate- polarity solvent ethyl acetate. The overall study of the fractions clearly showed that the activity was concentrated more in the polar fractions.

It is pertinent to further study the fractions and metabolites of this sponge species, which possess significant properties as bioactive compounds and have potential for use as anti-bacterial agents against these bacterial pathogens.



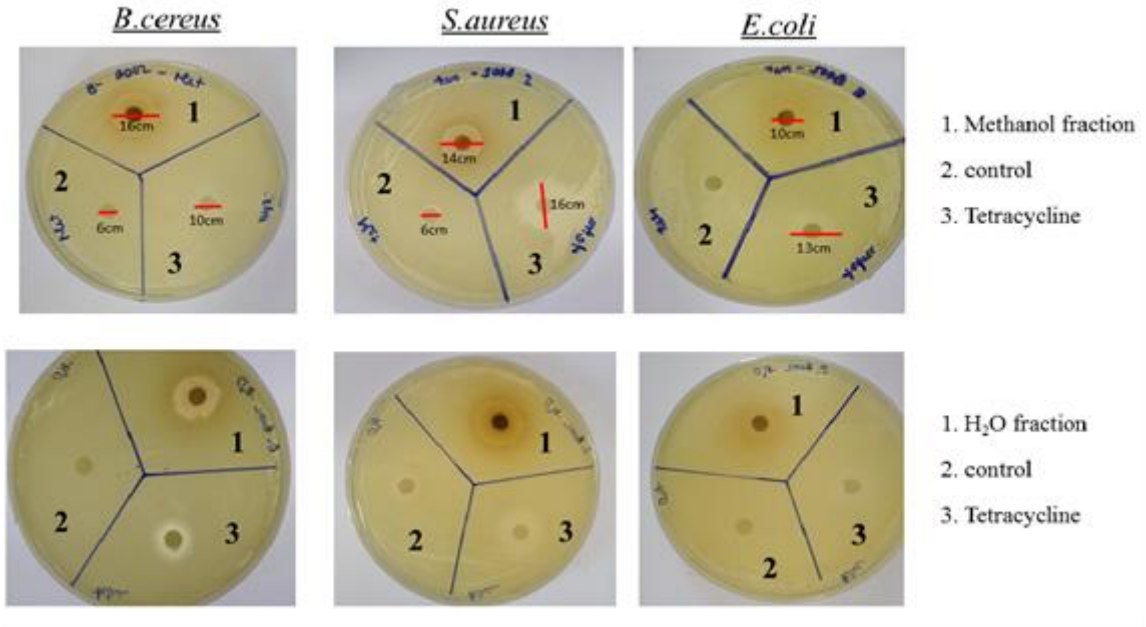


Figure 2. Anti-bacterial activity of *A.Suberitoides* extract fractions against the microbacterial following agar-well diffusion method

E. coli, *S. aureus* and *B. cereus*. were treated with *A.Suberitoides* extract fractions including n-hexane, ethyl acetate, water, and methanol. Tetracycline was used as positive control. Data presented are the means of three replicates. Values are expressed as mean±SD of three replicates

4. Conclusion

The results of the present study indicated that extracts of *A. suberitoides* exhibited anti-microbial activity against *E. coli*, *S. aureus* and *B. cereus*. in concentration- dependent manner and the majority of the active compound was found in the polar fractions. Further data need to be collected in the future to find out the name of active compounds which present in the polar fractions■

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