

Antimicrobial Activities of Marine Sponge Using Bioassay-direct Fractionation and Contact Bioautography Technique

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Previous report indicated that sponge voucher number (1L82008) possessed the highest antimicrobial activity among four sponges and three corals species investigated [1]. A large amount of sponge tissue (287g dry weight) was extracted with methanol and followed with water. The methanol and water extract were examined against two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus anthracis*), two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi strains (*Candida albicans* and *Cryptococcus neoformans*) using disc diffusion method.

The results showed that methanol extract had stronger antimicrobial activity than water extract (maximum activity was 19.5mm against *B. anthracis*, 400µg/disc). Obviously, gram-positive bacteria were more sensitive than gram-negative bacteria and fungus strains. *B. anthracis* (19.5mm, 400µg/disc) was the most sensitive bacteria while *Pseudomonas aeruginosa* (0mm) was the most resistant bacteria.

Since the methanol extract was found to be active, it was used for further bioassay-directed fraction in order to isolate the active compounds. From the bioassay results, dichloromethane (24mm, 400µg/disc) and n-butanol (18mm, 400µg/disc) fractions were found to be active. Purification of n-butanol fraction was done by using normal phase column chromatography (silica gel 70-230) eluted with n-butanol:acetic acid:water (12:3:5). Based on thin layer chromatography (TLC) profiles, the same fractions were collected, concentrated and assayed for antimicrobial activity against *B. anthracis*. The results showed that fractions (87-123) and fractions (124-183) were the most active fractions. The TLC of the active fractions were then developed using the same mobile phase and subjected to contact bioautography against *B. anthracis*, which showed inhibition zone at different retention time, indicating the presence of antimicrobial components.

Based on the observation, it could be concluded that the bioassay-guided fractionation and bioautography of 1L82008 marine sponge may come up with potent bioactive antibiotics. Research is still in progress to isolate the active compounds from n-butanol fraction as well as from dichloromethane fraction.

References

Haitham N. Qaralleh, Syed Z. Iddid, Shabudin Saad and Deny Susanti, *Programme and abstract book: 23rd Malaysian Society of Pharmacology and Physiology Scientific Meeting*, 12-13 May 2009, Kuala Lumpur, 92