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Microfractionation-based approach to screen potential specialized anti-microbial metabolites of Sri Lankan marine sponges

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The discovery of bioactive specialized metabolites from marine invertebrates has increased significantly during the last two decades. Marine sponges (phylum Porifera) are the existing oldest metazoan group that host rich microbial communities, and are considered as potential sources of future drugs in pharmacological and biomedical industry as they produce an impressive array of anti-inflammatory, immunosuppressive, neurosuppressive, antitumor, antiviral, and antimicrobial compounds. Of the few studies that have been carried out on such products in Sri Lankan marine sponges, the discovery of bioactive peptides has been largely overlooked. Therefore, a study was carried out to screen potential specialized anti-microbial metabolites especially peptides and sterols in Sri Lankan marine sponges with the intention of large scale isolation of targeted bioactive molecules. Bioassay-guided micro fractionation is an efficient method for the rapid screening of a large number of samples using only a small amount of starting material. Aqueous extracts (60% methanol) and organic extracts (Dichloromethane: methanol 9:1) of 20 different sponge species collected in coastal waters in Sri Lanka were micro fractionated into 48 fractions in deep well plates (1 mL per well), using reversed-phase HPLC. A volume of 100 μ L from each fraction was tested for antimicrobial activities and the wells with antimicrobial activity were analyzed by LC-MS coupled to a PDA detector to identify the molecular ions of the bioactive compounds. Of all the 40 extracts, 18 extracts produced by *Rhabdastrella globostellata*, *Aciculites* sp., *Rhabderemia* sp., *Erylus* sp., *Aulospongus* sp., *Manihinea* sp., *Agelas* sp., *Phakellia* sp., *Topsentia* sp., four species belonging to order Haplosclerida and one species belonging to order Suberitida showed bioactivity against *Staphylococcus aureus* (ATCC 25928). *Stylissa massa* showed bioactivity against *Escherichia coli* (ATCC 35218) while *Aulospongus* sp., showed bioactivity against *Candida albicans* (ATCC 90028). Sponge species were identified using their morphology, spicule arrangement and DNA barcoding. The 5' end of CO1 and 28S rRNA genes were sequenced for both forward and reverse direction. Sequences were compared using the BLASTn tool with the NCBI database in order to narrow down and identify the possible lowest taxonomic level. The wells that indicated bioactivity contained fractionated extract at ≤ 0.2 mg/mL concentration. Organic extracts of *Aciculites* sp., *Topsentia* sp. and the aqueous extract of *Erylus* sp. were the most active at ≤ 0.05 mg/mL. The targeted masses obtained from LC-MS will be isolated in large scale and the structural characterization of isolated specialized anti-microbial metabolites will be determined using LC-MS and NMR. Furthermore, these micro fractions will be subjected to lymphoma cell toxicity assay to identify potential anticancer compounds.

Keywords: Sri Lankan sponges, Specialized microbial metabolites, Antimicrobial activity

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