

PERMANENT REFERENCE



Taxonomic identification,
immunopharmacological and
toxicological study of
Haliclona (Soestella) sp, a marine
sponge species from Sri Lanka

A thesis submitted for the Degree of Doctor of
Philosophy



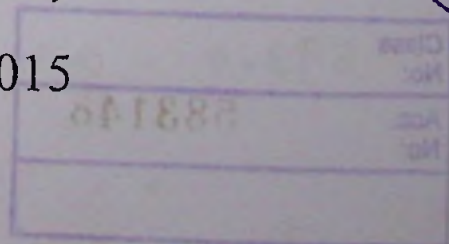
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Abstract

Despite the intense efforts by scientists, most drugs that are required to treat human diseases still remain undiscovered. Marine organisms are a remarkable source of secondary metabolites that are biologically active molecules with potential therapeutic usage. Marine sponges are ranked on top of the hierarchy of marine organisms with bioactive secondary metabolites. Marine environments were systematically investigated over the last 50 years and at present research is ongoing for systematic identification and drug discovery from marine organisms in general, and more specifically from marine sponges. The present study investigated the immunomodulatory activity of a few selected Sri Lankan marine sponges, with a comprehensive immunopharmacological study on *Haliclona (Soestella)* sp, identified presumably as a novel marine sponge species from Sri Lankan marine waters.

The vast marine sponge diversity of Sri Lanka led to the taxonomic identification of some selected sponge species, using spicule and skeleton morphology using light and scanning electron microscopy (SEM). A total of 11 sponge species of Class Demospongiae were identified and classified. This is the first report on the use of SEM for sponge identification in spongology in Sri Lanka.

Immunomodulatory activity of 4 Sri Lankan marine sponges were reported, including that of *Haliclona (Soestella)* sp. Crude sponge extracts of *Dyctionella conglomerata*, *Fasciospongia ondatjeana*, *Stylissa carteri* affirmed immunomodulatory activity with respect to non functional and functional immunological measures. Nevertheless, *Haliclona (Soestella)* sp was selected for the comprehensive immunopharmacological and toxicological study, due to its abundance. A number of *in vitro*, *ex vivo* and *in vivo* tests were performed to investigate the immunomodulatory effects of the *Haliclona (Soestella)* sp. sponge crude extract (HSCE). Antioxidant activity of the HSCE was tested using a few radicals *in vitro* and *in vivo* nitric oxide (NO) radical scavenging assay. Further, zoo chemical analysis of the HSCE resulted in the presence of alkaloids saponins and amino acids, followed by bioassay guided fractionation to isolate the bioactive compounds.

Immunomodulatory activity of the HSCE revealed immunosuppressive, immunostimulant and acute anti-inflammatory properties. Immunosuppression was evident in the following tested parameters: *in vivo* WBC, WBC DC, platelet, bone marrow cell and splenocyte counts, *ex vivo*

immune cell proliferation and cytokine production. Nonetheless, the HSCE showed immunostimulation with respect to *in vitro* phagocytic capacity of peritoneal macrophages. The HSCE showed potent anti inflammatory activity against acute phase oedema as revealed by the Carrageenan induced paw oedema and suppression of pro inflammatory cytokine production. Both anti radical activity on quenching DPPH radicals and *in vivo* NO scavenging activity of the HSCE established its antioxidant properties. Toxicological evaluation of the HSCE demonstrated that it was devoid of general toxicity, and hepato and renotoxic effects. Conversely, the HSCE was moderately haematotoxic and stressogenic. Nevertheless, the HSCE showed marked cytotoxic effect by inhibition of proliferation of the human larynx carcinoma cell line, Hep2, at a IC₅₀ value of 19.63 µg/mL, manifesting notable anticancer activity. The chloroform fraction of the HSCE also showed anti proliferative activity of Hep2 at IC₅₀ value of 29.52 µg/mL. Therefore, this fraction of the HSCE warrants LC-MS and NMR analyses for chemical characterization and structure elucidation of this anti cancer compound.

In conclusion, *Haliclona (Sostella)* sp., presumably a novel Sri Lankan marine sponge species, demonstrated immunosuppressant, immunostimulant, acute anti-inflammatory, antioxidant and anti-cancer properties with associated moderate haematotoxic and stressogenic effects. This prototype, comprehensive study on immunopharmacological and toxicological activity of a marine sponge species forms the baseline for future pharmacognosy studies on Sri Lankan marine organisms .