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**CHEMISTRY AND BIOLOGICAL ACTIVITY STUDIES
OF
SOME MARINE ALGAE AND THEIR ENDOPHYTIC FUNGI**

A THESIS PRESENTED

BY

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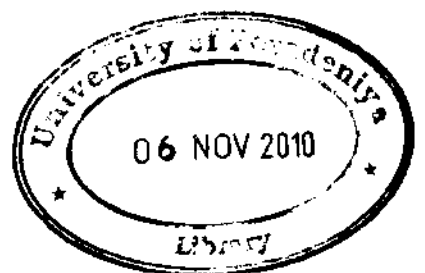
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**CHEMISTRY AND BIOLOGICAL ACTIVITY STUDIES
OF
SOME MARINE ALGAE AND THEIR ENDOPHYTIC FUNGI**

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ABSTRACT:

Present investigation is initiated with the aim of identifying biologically active and economically important natural products from Sri Lankan marine algae (seaweeds) and their endophytic fungi. The work embodied in this thesis has been divided into two parts. **Part-A** contains chemistry and biological activity studies of some Sri Lankan seaweed extracts that have been collected from different locations of Sri Lanka. The **Part-B** of the thesis deals with chemistry and biological activity studies of endophytic fungi derived from some Sri Lankan seaweeds.

Part-A:

In the present study, forty six fresh marine algal species were collected from the coastlines of Sri Lanka, and identified. Methanol extracts of seaweeds were subjected to antibacterial, antifungal, antioxidant, cytotoxic and seed germination inhibition activity assays. While none of the seaweed extracts showed activity against tested bacteria and fungi, some of the extracts showed activity against brine shrimp lethality bioassay and seed germination inhibition assay. Of the 24 seaweed species examined for the cytotoxicity, 9 showed LC_{50} value below 1000 $\mu\text{g/mL}$ against brine shrimp lethality assay. Above cytotoxic seaweed extracts could be further investigated for potential anticancer/antitumor activities. Sixteen seaweed extracts were tested for lettuce seed germination inhibition assay and seven of them showed significant reduction in the average radicle length of lettuce seeds, showing their allelopathic activity. Of them

Caulerpa racemosa (Forsskål) J. Agardh showed the lowest % germination value (17.5%) in the lettuce seed germination assay. At the same time *Caulerpa sertularioides* extract which gave a 25% germination value appeared to possess the smallest average radicle length of 0.63 ± 0.19 cm. Above allelopathic active seaweeds could be used as environment friendly natural herbicides. Interestingly, *Caulerpa racemosa* f. *laxa* showed seed germination stimulant activity with the highest % germination value (90%) and longest average radicle length of 3.83 ± 0.11 cm. These results showed *Caulerpa racemosa*'s potential as a bio-fertilizer.

Methanol extracts of red algae, *Laurencia hetroclada*, *Amphiroa anceps* and green alga *Ulva lactuca* were chemically investigated. This investigation resulted in the isolation and structure elucidation of 12 pure compounds. Arugambenol, from *Ulva lactuca* and two brominated sesquiterpenes from *Laurencia hetroclada*, proved to be new. The known compounds algaane-1, cholesterol, caulerpin, isofucosterol, triglyceride with two linoleic acid molecules and one stearic acid, oleic acid, sucrose, β -sitosterol and stearic acid were also isolated from above extracts. The compound caulerpin, showed a significant suppressive effect on polymorphonuclear neutrophils (PMNs) and T-cell proliferation inhibition assays.

Part-B:

Specimens collected from seaweeds were used to isolate endophytic fungi. Ten endophytic fungal strains were isolated from 10 algal samples. Two fungal strains isolated from red alga, *Laurencia ceylanica*, and brown alga, *Dictyota kunthi*, were chosen for the large scale cultivation on Czpex Dox liquid medium, to investigate their secondary metabolite productions. This investigation resulted in the isolation and structure elucidation of 12 pure compounds including a new butyrolactone. The new butyrolactone showed significant activity against immunomodulatory and T-cell proliferation inhibition assays. It also showed antioxidant activity and the β -glucuronidase enzyme inhibitory activity. The known butyrolactone-1 showed significant activity against immunomodulatory, T-cell proliferation inhibition, antioxidant, β -glucuronidase and phosphodiesterase inhibition activity studies.

The previously reported compound (+)-asterrelenin also showed significant inhibition activity against β -glucuronidase enzyme while (+)-terrein, showed potent inhibition activity against T-cell proliferation and the urease inhibition assay. Additionally, (-)-prolylleucyldiketopiperazine and genistein were also isolated from endophytic fungal strain obtained from brown alga, *Dictyota kunthi*. The compound (-)-prolylleucyldiketopiperazine showed a significant inhibition activity against β -glucuronidase enzyme.

The above chemical investigations and bioassay results indicated that Sri Lankan seaweeds and their endophytic fungi are very good sources of potential bioactive compounds. *Galaxaura lapidescens*, *Sargassum* sp.-1, *Dictyota* sp.-1 and *Dictyota* sp.-2 caused brine shrimp deaths below the level of positive control, and it is a strong indication of presence of potential anticancer/antitumor active compounds and therefore, further studies should be carried out on them. *Ulva fasciata*, *Cladophora* sp., *Caulerpa racemosa* (Forsskål) J. Agardh, *Caulerpa sertularioides*, *Amphiroa anceps*, *Gracilaria hikkaduensis* and *Jania* sp. have shown statistically significant seed germination inhibition activity, and they could be used as the sources of natural herbicides. *Caulerpa racemosa* f. *laxa* extract has shown seed germination stimulant activity and this seaweed could be used as a fertilizer. Further, above investigation suggested that *Ulva lactuca* is non-cytotoxic and its methanol extract contained fatty acids such as linoleic acid and oleic acid; and free sugars along with other compounds. Therefore, the presence of linoleic acid and free sugars in *Ulva lactuca* further highlighted the nutritional value of this seaweed, which is widely consumed in the Far East. *Ulva lactuca* is popularly known as sea lettuce, and it is an excellent idea to introduce this nutritious seaweed to the Sri Lankan diet, as it is naturally abundant in the Sri Lankan coastal line.