Young Scientists' Circle

Bioprospecting Endolichenic Fungi from lagoon associated mangrove ecosystems in Sri Lanka; An Untapped Treasure Trove for Discovery of Special Structures and Bioactive Compounds

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Natural product chemistry has kept researchers on toes for many years owing to its distinctive caliber in providing new hopes to quench necessities of mankind. The uncovered metabolites have served in many aspects such as in food industry, energy generation, fabric production, and most importantly, drug discovery. Even though the focus of discovering new natural products mainly lingered around higher organisms such as plants, the discovery of penicillin from *Penicillium notatum* by Alexander Fleming in 1928 immensely shifted the focus towards microorganisms as more prominent prospects[1]. The recent developments in discovering bioactive secondary metabolites from microorganisms have reached a peak due to the easiness in handling and minimum impact on the loss of biodiversity when obtaining sources. Hence lichens, inevitably, draw attention due to being a symbiotic product of such three Kingdoms; algae from Kingdom *Protista* and/or cyanobacteria from Kingdom *Monera* as the photobiont and filamentous fungi from Kingdom *Mycota* as the mycobiont. In my research, fungi which live asymptomatically on the thalli of this fascinating micro-environment, thus named endolichenic fungi (ELF), are explored in order to find new bio-active molecules. Since the initiation of the discovery of heptaketides from *Corynespora* sp. inhabiting the Cavern Beard Lichen, *Usnea cavernosa* conducted by my supervisor Prof. Priyani Paranagama[2], the interest towards exploiting these captivating group of microorganisms have grown all over the world. So far such bio active compounds uncovered from ELF exceeds 176 in number[3]. Sri Lankan records of polyketides obtained from ELF *Penicillium citrinum* and *Curvularia trifoli* isolated from lichens *Parmotrema* sp. and *Usnea* sp. respectively, also are included in the said listing[4,5]. The attention towards the ELF inhabiting the lichens found in mangrove eco-systems was inspired due to the assumption that the constantly changing and challenging environment to which the ELF are exposed can induce them to produce remarkable secondary metabolites. In the study conducted using the ELF inhabiting the lichens collected from mangrove associated plants of Puttalam lagoon[6], some promising compounds were discovered, which included a cytotoxic compound from the ELF *Xylaria psidii* isolated from the lichen *Amandinea medusulina*[7] along with two anti-oxidant and anti-inflammatory compounds from *Neurospora udagawa*, two cytotoxic compounds from *Talaromyces pinophilus* and an antibacterial compound from *Lasiodiploidia pseudotheobromae* (in the process of being published). With such proven evidence, moving the next focus to the mangrove ecosystem of Negombo Lagoon was almost inevitable. The collection of lichens obtained from the "Kadol Kale" area of Negombo lagoon, which also is the first study of lichens conducted in the said area, consisted of 31 lichens. Following the identification of lichens conducted with the assistance of Dr. Gothamie Weerakoon (Senior Curator, National History Museum, London), it was found that the majority of the samples belonged to the Crustose category, which included *Pyrenula ochraceoflava, Bactrospora myriadea, Graphis librata, Graphis furcata, Arthonia parantillarum, Arthonia antillarum, Opegrapha medusulina* and *Graphis caesiella*. The Foliose lichen *Pyxine cocoes* and Fruticose *Roccella montagnei* completed the variety of the collection. The total of 65 ELF, isolated from the aforementioned lichens, belonged to 26 different species after being identified using rDNA-ITS region sequence homology to the GenBank accessions under the supervision of Dr. Renuka Attanayake (Senior Lecturer, University of Kelaniya). Ethyl acetate crude extracts of these identified ELF were then used to screen for different bio activities. For this purpose, several bio assays were conducted to screen anti-oxidant, anti-inflammatory, tyrosinase inhibitory and anti-bacterial activities of the crude extracts. All the

extracts ultimately showed at least one of the potencies screened for, while some even surpassed the capacities of the positive controls they were compared with. Namely, the crude extracts of the ELF *Hypoxylon anthochroum, Hypoxylon lividipigmentum, Curvularia lunata, Chaetomeum globusum* showed anti-oxidant activity on par with the commercially available antioxidant butylated hydroxytoluene (BHT) and the same extracts showed comparable anti-inflammatory activity analogous to Aspirin which is in use as a remedy for inflammation. While showing anti-inflammatory activity, *Xylaria feegenesis* exhibited antibacterial activity for the three aerobic bacterial strains *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Moreover, the extract of *Curvularia lunata* showed superior anti-bacterial activity, not only against the aforementioned aerobic bacterial strains but also against the anaerobic strain *Streptococcus mutans* (critically responsible for tooth decay) even exceeding the potency of chlorohexidine found in the market. In order to identify the real heroes behind these amazing abilities, many of the bioactive compounds have been and are being isolated using chromatographic techniques. With the collaboration of The National Institute of Pharmaceutical Education and Research, Ahmedabad, India of the Indo-Sri Lanka grant (funded by the Ministry of Science Technology and Research), the spectroscopic facilities needed to elucidate the structures of the isolated compounds will thankfully be fulfilled. The mutual exchange of knowledge and facilities between the two countries have immensely benefitted both the parties alike.

Almost at the last phase of my project, I'm eagerly waiting to be a contributor to the pharmaceutical industry at the inceptive level by providing them with a few novel bio active compounds to bring to the market. With crossed fingers, I sincerely hope that they will be able to give better curing abilities with less side effects or become solutions to overcome issues such as antibiotic resistance. No matter how many medicines are already in use, the need for new drugs will never be extinguished. Hence, the search for new bioactive compounds from natural sources will never go out of trend. And I'm humbly proud to be an active researcher adding to the knowledge pool of this unceasing demand of drug discovery.

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