Chandrasena Memorial Award

Awarded for an exceptional research contribution of an original nature in the field of Organic Chemistry and/or related areas such as Biochemistry, Pharmacognosy, Molecular Biology and Bioactivity studies.

Chandrasena Memorial Award - 2019

Dr. Pamoda Ratnaweera is a Senior Lecturer attached to the Department of Science and Technology at the Uva Wellassa University of Sri Lanka. She obtained her BSc Special Degree in Zoology with first-class honors and three gold medals having topped the batch at the University of Colombo in 2007. She got a Government HETC scholarship for her postgraduate studies and obtained her PhD in Natural Products Chemistry also from the University of Colombo, in 2015. Dr. Ratnaweera has served as a faculty member for over a decade at the Faculty of Applied Sciences, Uva Wellassa University, Sri Lanka. She also served as a Visiting Scientist at the Department of Chemistry, University of British Columbia, Canada, in 2012, 2014 and 2016. Dr. Ratnaweeera was the recipient of the prestigious General Research Committee Postgraduate Research Award of the Sri Lanka Association for the Advancement of Science (SLAAS) in 2016 and Vice Chancellor's Award for the most outstanding young researcher of the year 2017 by the Uva Wellassa University. Dr. Ratnaweera possess over 16 research publications in peer-reviewed indexed journals and two patents for her credit. She received Presidential Awards for her high-quality natural product research publications presented in some prestigious journals. Dr. Ratnaweera's research currently conducted at the Uva Wellassa University is funded by the National Science Foundation Sri Lanka and through a University research grant. Beside them, she has won the Royal Society Commonwealth Grant, UK in 2016 to work on a collaborative research project in a biosynthetic enzyme laboratory at the University of British Colombia, Canada.

Novel and interesting antibiotic scaffolds from endophytic fungi of Sri Lankan origin

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Antibiotic resistance within a wide range of infectious agents is increasing steadily, causing a growing public health crisis in the world today. The World Health Organization (WHO) has reported infectious and parasitic diseases as a leading cause of death in the world, causing 15.6 % of all deaths in women and 16.7 % in men. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have currently become a global pandemic in hospitals, long-term care facilities and community health settings.

The development of resistance by pathogens limits the useful lifespan of antibiotics, thus causing an urgent need for introduction of new compounds. The number of new antibiotics reaching the market has also decreased over the past 25 years. Scientists have found the antibiotics evolved from natural products can penetrate the barriers of target bacteria more successfully than antibiotics developed from synthetic approaches. Hence, the most appropriate method to address the antibiotic resistance of bacteria is to find new alternatives to the currently available broad spectrum antibiotics through exploitation of nature for novel compounds.

Fungal endophytes are a group of microorganisms which spend all or part of their life cycle inter and/or intra-cellularly colonizing the healthy tissues of a plant without causing any visible manifestation of disease symptoms. They are an innovative group of organisms that can produce a plethora of secondary metabolites that feature unique structural characteristics and fascinating biological activities. However, only a handful of plants on the earth have been studied so far for endophytic fungal metabolites. Therefore a worldwide scientific effort is currently under way in isolating fungal endophytes and their bioactive natural products for a better and healthier future.

Due to the vast number of plant species in the world, creative and imaginative strategies are necessary to quickly narrow down the search for bioactive endophytes. This provides the best opportunities to isolate endophytes prone to produce novel bioactive products. According to Strobel, plants from distinct environmental settings and/or with an unconventional biology, are considered to be a promising source for isolating novel fungal endophytes as well as new secondary metabolites.

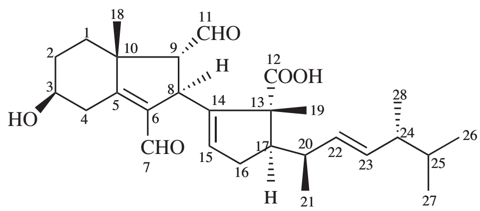
Sri Lanka is a relatively small island with a variety of climatic conditions and a high degree of biodiversity that may harbor endophytes with distinctive biosynthetic abilities. In the backdrop that effective and innovative antibiotics are needed to replace older ones, which are becoming obsolete due to drug resistant pathogenic bacteria, exploring the antibacterial producing capacity of endophytes becomes meaningful. So far, only a few Sri Lankan plants have been systematically investigated for the production of antibacterial substances by their fungal endophytes.

During our hunt of antibiotics and other bioactive compounds, endophytic fungi from various plants from different ecological settings in Sri Lanka were isolated, screened, the major bioactive secondary metabolites were purified using bioassay guided chromatographic techniques, isolated bioactive compounds were characterized using NMR and mass spectroscopic techniques and bio activities were evaluated. In the case of the novel compound solanioic acid, confirmation of correct structural/stereochemical assignments was acquired by X-ray crystallography, and semi-synthetic modifications while biogenesis pathway was investigated through stable isotope feeding experiments.

Investigation of weed plants commonly refereed as grasses and sedges led to the most interesting finding within our endophytic fungal research. Weed plants show vigorous and aggressive growth regardless of environmental or ecological conditions. They successfully grow in harsh and disturbed environments and have the ability to flourish despite insect, microbial or pathogenic attacks. These considerations led to the hypothesis that endophytic fungal populations inhibiting these plants, via the production of certain specific biologically active secondary metabolites, may contribute towards the hosts' ability to overcome biotic and abiotic stresses. This was supported by our recent isolation of the novel antibiotic solanioic acid which possesses a highly functionalized and rearranged steroidal carbon skeleton from *Rhizoctonia solani* isolated from the medicinal weed *Cyperus rotundus* (Kaladuru) common sedge in Sri Lanka.

Solanioic acid (**1**) has an unprecedented carbon skeleton that features a highly functionalized conjoint ring system which represents a new antimicrobial scaffold, with promising in vitro activity against the problematic human pathogen Methicillin resistant *Staphylococcus aureus* (MRSA, MIC: 1 μg mL^{-1}). It also showed activities against Gram positive *Bacillus subtilis* (MIC: 1 μg mL^{-1}), *Staphylococcus aureus* (MIC: 1 μg mL^{-1}) and the yeast *Candida albicans* (MIC: 16 μg mL^{-1}). The promising activity of this compound against MRSA warrants further investigation of solanioic acid as an antibacterial drug lead (Patent no.18450).

The culture feeding experiment with [1-^{13}C]-acetate, [2-^{13}C]-acetate and [1,2-^{13}C]-acetate showed that the steroid ring B contraction involved in the biogenesis of the unprecedented carbon skeleton of the solanioic acid involves cleavage of the C-5/C-6 bond. Besides, the [1-^{13}C]-acetate feeding study yielded not only solanioic acid but also its C-9 epimer (**2**). The isolation of 9-epi-solanioic acid suggests that first natural product formed by this pathway is 2, which have the normal C-9 steroid configuration. The 9-epi-solanioic acid is then either biosynthetically or spontaneously epimerized to solanioic acid which is the thermodynamically most stable epimer.



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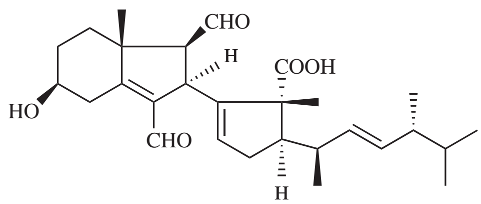

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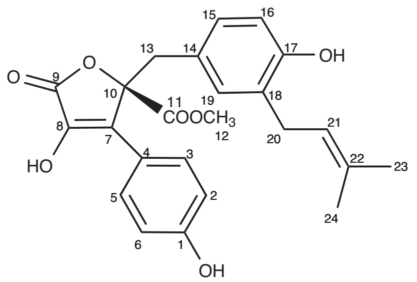
Apart from solanioic acid, Butyrolactone I (**3**) was isolated from the endophyte Trichoderma virens from the sedge Cyperus melanosperrmus and 9-epi viridol (**4**) from endophyitc Aspergillus terreus from Cyperus bulbosus. The MIC values of Butyrolactone I and 9-epi viridol were in the range 128-256 μg mL^{-1} against Gram-positive *B. subtilits*, *S. aureus*, MRSA and *Escherichia coli*.



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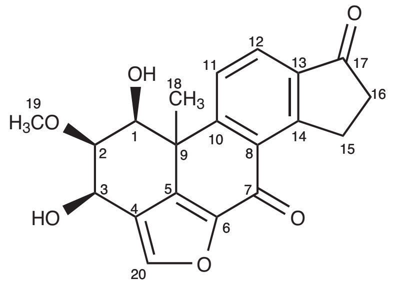

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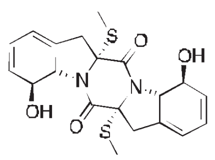
Costus speciosus (Tebu) is a traditional indigenous medicinal plant in Sri Lanka reputed to control diabetes. Three new thiodiketopiperazine derivatives, rostratazine A (**5**), rostratazine B (**6**) and rostratazine C (**7**) along with exserohilone (**8**) and boydine A (**9**) were isolated from the endophyte *Setosphaeria rostrata* obtained from Costus speciosus collected from a home garden. The compound 8 showed alpha-glucosidase inhibitory activity (IC_{50} value 82 μg/mL) while 6 showed porcine pancreatic alpha amylase inhibitory activity (IC_{50}: 250 μg/mL).



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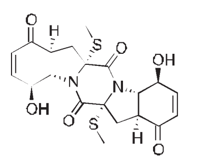

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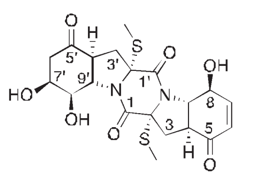

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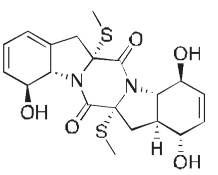

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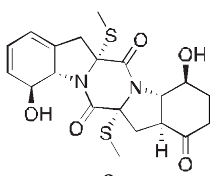

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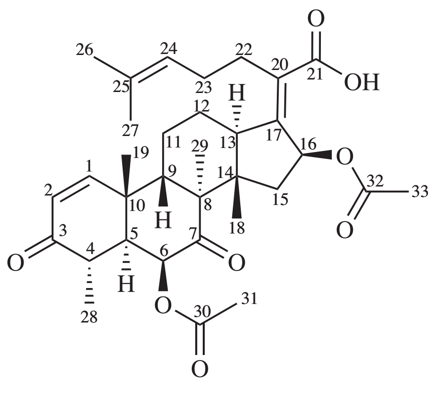
Rainforests due to their high species diversity and competition become potentially productive environments for discovery of novel molecular structures and biologically active metabolites. *Anoectochilus setaceus* (Wana raja) is an endemic endangered orchid traditionally used for snake bite poisoning. We isolated a nortriterpenoid, helvolic acid (**10**) from an endophytic *Xylaria* sp. obtained from the orchid *Anoectochilus setaceus* collected from Kanneliya rainforest Sri Lanka Helvolic acid reported antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA, MIC 4 μg mL^{-1}) and *Bacillus subtilis* (MIC: 2 μg mL^{-1}).

Opuntia dillenii (Katu pathok) is an invasive cactus found in the South-Eastern province of Sri Lanka. Shipunov *et al.* have mentioned that in the host's invaded range, endophytes increase the competitiveness of the host by producing metabolites inhibitory to evolutionarily native plants. In our investigation endophytic *Fusarium* sp. isolated from the invasive cactus Opuntia dillenii yielded the antimicrobial secondary metabolite equisetin (**11**). Equisetin, is a tetramic acid derivative and it showed MIC values of 8 μg mL^{-1} against *B. subtilis* and 16 μg mL^{-1} against *S. aureus*. We proposed these biologically active substances may enhance the competitive ability of the host against microorganisms and perhaps increase its adaptability to withstand the biotic and harsh abiotic stress factors that assist in the successful establishment of *O. dillenii* to the detriment of native plants in the area.



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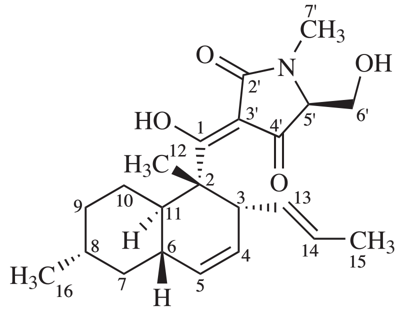

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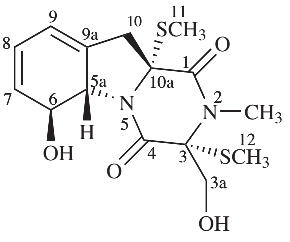
Mangrove associates are species mainly distributed in terrestrial or aquatic habitat but also occur in the mangrove ecosystem. According to Tomlinson criteria, mangrove associates are also distinguished from true mangroves by lacking aerial roots, vivipary and no physiological mechanism for salt exclusion. However, mangrove associates growing in the mangrove habitat also have to face the same extreme ecological conditions as the true mangroves. Therefore these mangrove associates also have the potential of producing bioactive natural products as the true mangroves. This is evident by our isolation of antimicrobial gliotoxin (**12**) (MIC: 0.13 μg mL^{-1} against *B. subtilis*, 16 μg mL^{-1} against *S. aureus*, 32 against MRSA and *E. coli* and 64 μg mL^{-1} against Pseudomonas aeruginosa) and Bisdethiobis(methylthio) gliotoxin (**13**) from an extract of the endophytic fungus *Hypocrea virens* from the plant *Premna serratifolia* collected from mangrove habitat in Negombo.

Calamus thwaitesii is a vulnerable rattan species in Sri Lanka which is rapidly decreasing due to overexploitation for furniture industry. The endophytic Mycoleptodiscus species isolated from the leaves of C. thwaitesii resulted the alkaloid mycoleptodiscin B (**14**) which showed promising antimicrobial activities against *B. subtilis* (MIC: 0.5 μg mL^{-1}), S. aureus (MIC: 1 μg mL-1), MRSA (MIC: 32 μg mL^{-1}) and pathogenic fungus *Candida albicans* (MIC: 64 μg mL^{-1}). Though mycoleptodiscin B was previous reported to possess moderate cytotoxic activity our study was the first to report the antimicrobial activities of mycoleptodiscin B (Patent No. 18931)



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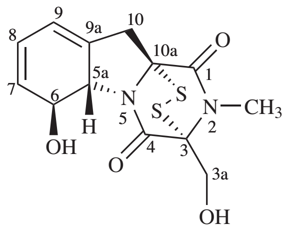

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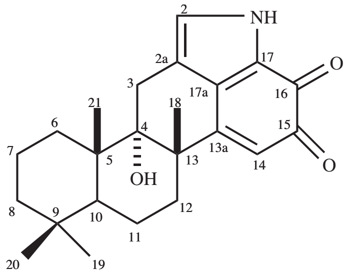

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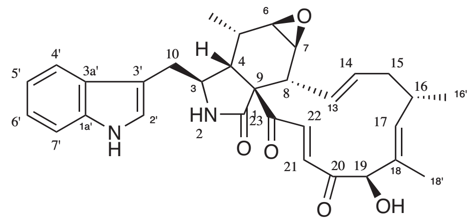
Inland fresh water bodies also are productive ecosystems in the world which house diverse microorganisms. Aquatic plants highly adapted to its environmental and ecological conditions also harbour endophytic fungi having bioactive metabolites. Our investigation of endophytic fungi of *Nymphaea nouchali* (Nil manel) led to the isolation of the known secondary metabolites chaetoglobosin A and C (**15, 16**) from *Chaetomium globosum*, with chaetoglobisn A showing good antibacterial activities (MIC: 16 μg mL^{-1} against *B. subtilis*, 32 μg mL^{-1} *S. aureus* and MRSA).

Except solanioic acid (**1**), 9-epi-solanioic acid (**2**) and rostratazines (**3-5**), other metabolites isolated through this extensive investigation turned out to be previously known compounds. However, in some instances the fungal source was new while the isolation of endophytic fungi from the host organisms and report of their antimicrobial activities turn out to be novel reports with some ecological implications. The investigations revealed that endophytic fungi from different, harsh and competitive environment settings are capable of producing a variety of bioactive compounds which confirm Strobel's rationale that host plant selection from unique and unusual ecological settings may lead to the discovery of novel chemical scaffolds.



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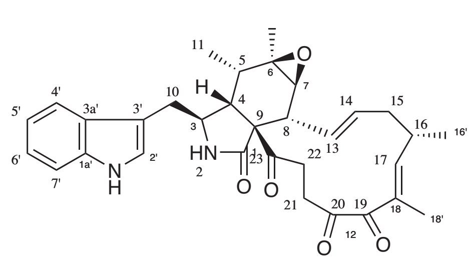

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This study is a clear indication that the endophytes of unique Sri Lankan organisms is a fruitful source for isolating novel bioactive structures such as solanioic acid which will be useful antibacterial agents and encourages further investigations into this potentially very productive field of study.

Acknowledgements

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Guest Articles

Nanopore: An Ostensibly Simple Sensor Stamping Single Molecule-Level Ohmic Readouts

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Single-molecule/particle level analysis has surpassed the era of ensemble average studies and now faces grander challenges unique to each molecular class, for example, genomic sequencing with minimal financial foot-print been the most notable. The eventual goal of most of these efforts is to create a hand-held device that can read data on-site, upload it to a cloud, perform analysis and provide feedback in minutes while having an accuracy comparable or surpassing that of conventional instrumentation. Nanopore sensors have emerged at a time of demand with the promise to tackle a wide spectrum of biomolecules to cater fields such as biomedicine, mechanical engineering, pharmaceutical chemistry, physics, *etc*. A nanopore in its simplest definition is a nanoscale aperture spanning an impervious natural or solid-state membrane which separates two electrolyte reservoirs. The analyte is added to one chamber (cis) and driven across the nanopore in response to a voltage bias applied to the other chamber (trans). The transiting analyte perturbs the ionic-current of the open-pore generally causing a drop in the current (exceptions exist)—more formally termed an event—which bares molecular information characterized by the duration (Δt), depth (ΔI) and inter-event duration (Δf). Charged molecules travel by electrophoresis (EP) and depending on the pH, the nanopore surface might have a net charge, generating an electroosmotic force (EO) that opposes or reinforces the electrophoretic force. Uncharged molecules may travel solely by electroosmosis. Manipulation and optimization of these forces (EP and EO) enable successful sensing.

The first demonstration of a nanopore to profile DNA was merely two decades ago using α-Hemolysin—a natural nanopore excreated by a bacteria—and since then, it has evolved into characterizing a plethora of biological and non-biological analytes—proteins, glycans, viruses, liposomes, exosomes, polymers. The focal point of nanopores since its inception has been on DNA sequencing. Natural pores were chosen because of their immaculate size, reproducibility and comparative dimensions of the sensing zone with nucleotide spacing being amongst other beneficial factors. Nanopore sensing is nondestructive, label-free and usually operate at a nM to pM concentration range at a bias of ≤ 1V, requiring only few microliters of the sample. While the technology associated with biological nanopores has expanded