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Cover Image: Artistic capture of a tree at Hanthana protected area. Hanthana is the home to a wide variety of flora and fauna, including a number of endemic species. The area is a 'natural laboratory', important especially for research in the fields of life and earth sciences.

Cover Design: Dr. A. K. Amarasinghe, Faculty of Science, University of Peradeniya.

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SRI LANKA

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MESSAGE FROM THE DIRECTOR

The Postgraduate Institute of Science (PGIS), is a national Institute attached to the University of Peradeniya and was established in 1996. It offers a number of Postgraduate programmes leading to a Postgraduate Certificate, Diploma, Master, M.Sc, M.Phil and Ph.D. degrees. The academic programmes of PGIS are conducted through 11 Baords of Study and it encourages advanced scientific research by providing facilities and a conducive environment for research and teaching so as to achieve the goals of the Institute and the wider society. Our students carry out their research in a range of laboratories established at the PGIS as well as at many other recognized research institutes in Sri Lanka and abroad. Our research areas extend over a wide range of disciplines, from basic to applied sciences.

The publication titled, "PGIS Postgraduate Research Highlights – 2020" comprises of findings of individual researchers who have received Ph.D and M.Phil. Degrees in 2019. Many of their findings have already been published in reputed journals and some researchers have also been honored with awards for their outstanding research.

I appreciate the dedicated service of Prof. Sanjeewa P.K. Malaviarachchi as the Editor-in Chief of this publication, and the members of his review group for their valued contribution. I express my heartfelt gratitude to all M.Phil and Ph.D holders of the PGIS and their supervisors for providing summaries of their valuable research and their consent to publish them.

Prof. H.M.T.G.A. Pitawala
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MESSAGE FROM THE EDITOR IN CHIEF

I am delighted to present the compilation of the PGIS Research Highlights 2020 with a great satisfaction as all the members of the Committee contributed immensely towards its success. I take this opportunity to thank all Ph.D. and M.Phil. graduates and their supervisors who have contributed to this volume, for the high quality research that has been undertaken by them.

This publication serves as an additional means of disseminating knowledge gained through postgraduate researchers who graduated during the period of 2019/20. Further information on topics that interest anyone who reads this publication could be obtained by contacting relevant authors or their supervisors. I believe, for those who want to embark on collaborative research either locally or internationally, this publication would be serving as a clear guide.

My gratitude is extended to the Postgraduate Institute of Science for giving every encouragement to these research activities and the Director, Prof. H.M.T.G.A. Pitawala, Chairperson of the RESCON, Prof. G.W.A.R. Fernando and all the members of the Editorial Committee of RESCON Research Highlights - 2020 for all the assistance given to me in compiling this publication. Finally, I extend my best wishes to RESCON 2020 for a successful completion of proceedings.

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Contents

TAXONOMY AND BIOGEOGRAPHY OF SRI LANKAN LEAFY LIVERWORTS (PHYLUM MARCHANTIOPHYTA, CLASS JUNGERMANNIOPSIDA)

B. M. S. K. Bandaranayake *graduated in 2016 with a B.Sc. special in Botany from the University of Peradeniya. Soon after she joined temporary academic staff at the Department of Botany University of Peradeniya. Subsequently she started her M.Phil. at the Postgraduate Institute of Science, University of Peradeniya and completed it in 2019. Her study represents the first detailed taxonomic survey of Sri Lankan leafy liverworts.*

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Liverworts including all bryophytes have formed an ecologically significant component of life on land for over 400 million years. They are the second largest embryophyte lineage next to angiosperms (Shaw and Goffinet, 2000). Liverworts play important roles in many ecosystems such as montane forests and rain forests. They are very sensitive to pollutants and other micro climatic changes like temperature and humidity within the habitat because they lack a proper cuticle, proper vascular system or roots as in vascular plants.

Liverworts, represent the earliest diverging lineage of extant land plants (Shaw and Goffinet, 2000) and within liverworts, leafy liverworts (Phylum Marchantiophyta, Class Jungermanniopsida) represent one of the most taxonomically complex groups. Leafy liverworts of Sri Lanka remain poorly studied compared to other groups of plants. Existing checklists of leafy liverworts are mostly based on collections made during British colonial period. Scarcity of detailed taxonomic studies, especially the lack of specimen-based studies and locality details of the existing taxa is a major obstacle in understanding the taxonomy and biogeographic patterns of this important group of plants in the country.

The main objectives of the present study were,

(I) To carry out a detailed taxonomic study on leafy liverworts of Sri Lanka: identify and document the taxa and to study their ecology

(II) To trace biogeographic affinities of the taxa identified during the study

(III) To identify threats imposed on leafy liverworts in order to implement necessary conservation measures

The outcome of the study will record a national level survey of the leafy liverworts of Sri Lanka.

Methodology

Fresh samples with vegetative and reproductive structures were collected from throughout the country covering all possible geographic localities. Collected specimens were studied for morphological, anatomical, and spore morphological characteristics and identified up to generic/specific level following most recent keys

and monographs. Taxonomic keys, photo plates and descriptions were prepared for all the identified taxa. Herbarium specimens were prepared using Schofield (1985) method. The distribution, habitats and locality details of the species were documented. Distribution maps were prepared using National Geographic Mapmaker Interactive and www.gbif.org. Biogeographical patterns were traced for all the leafy liverworts identified during the study following Zhang and Corlett, (2003).

Results and Discussion

Twenty-one families, 47 genera and 129 species of leafy liverworts were recorded including 6 new species records and one new genus record to Sri Lanka (Bandaranayake *et al*., 2020). Taxonomic keys were prepared for all the families, genera and species identified (figure 1). Thirteen categories of biogeographical patterns were identified for the leafy liverworts encountered during the study.

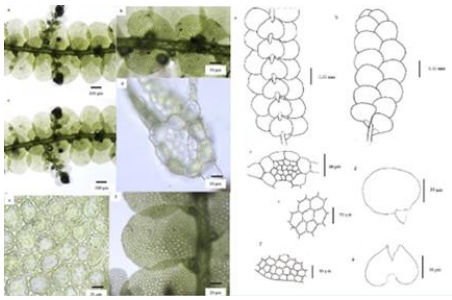

Figure 1. Photo plate and illustration of *Lejeunea sordida* (Nees) Nees

Conclusions

New specie and generic records and species that can be used as potential bio-indicators were indented. Results will contribute to the much needed "Flora of Bryophytes in Sri Lanka" and will increase the biodiversity figures of the country. Major habitats, ecology, and phenological of the species were documented. These data will contribute to the Red listing process of bryophytes in Sri Lanka.

Source of Funding

National Science Foundation (Grant No: RG/2016/EB/01).

Selected References

Schofield, W.B. (1985) *Introduction to bryology*. Macmillan Publishing Company. London.

Shaw, A. J. and Goffinet, B. (2000). Bryophyte biology. Cambridge University Press.

Zhang, L., and Corlett, R. T. (2003). Phytogeography of Hong Kong bryophytes. Journal of biogeography, 30(9), 1329-1337.

Publications

Bandaranayake, B. M. S. C., Ruklani, N. C. S. & Rubasinghe, S. C. K. (2020). New additions to the leafy liverwort flora (Marchantiophyta, Jungermanniopsida) of Sri Lanka. J.Natn.Sci.Foundation Sri Lanka 2020, 48 (2), 199 – 209.

Bandaranayake, B. M. S. K. & Rubasinghe, S. C. K. (2018). Leafy liverworts in Sinharaja and Kanneliya rain forests. Sri Lanka Forester, 39, 37–48.

Bandaranayake, B. M. S. K. & Rubasinghe, S. C. K. (2018). Leafy liverworts in Sinharaja and Kanneliya rain forest. Proceedings of the Research Symposium on Wet Zone Forests. Forest Department, Ministry of Mahaweli Development and Environment, 16.

Bandaranayake, B. M. S. K. & Rubasinghe, S. C. K. (2018). Diversity and conservation of little-known leafy liverworts of Sri Lanka. Proceedings of PGIS Research Congress, 05, 73.

Bandaranayake, B. M. S. K. & Rubasinghe, S. C. K. (2017). A Taxonomic survey of leafy liverworts in Sri Lanka (Phylum Marchantiophyta, Class Jungermanniopsida). Proceedings of the Peradeniya University International Research Sessions (iPURSE), 21, 457.

Thesis Reference

Bandaranayake B M S K. (2019). Taxonomy and Biogeography of Sri Lankan Leafy Liverworts (Phylum Marchantiophyta, Class Jungermanniopsida). M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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CHEMICO-GEOGRAPHY OF SELENIUM AND ITS IMPACT ON FOOD CHAIN QUALITY AND ANIMAL HEALTH IN SRI LANKA

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Selenium (Se) is a naturally occurring metalloid element, which is essential to human and other animal health in trace amounts, but is harmful in excess. Se has a narrow range between dietary deficiency and toxic level thus the intakes by humans and animals should be carefully controlled. Se deficiency is a known contributing factor for the prevalence of goiter (Fordyce *et al.,* 2000) and Chronic Kidney Disease of uncertain etiology (CKDu) (Jayatilake *et al.,* 2013) in the Sri Lankan population. The main objective of this study was to investigate the Se distribution in the environment of Sri Lanka to understand and evaluate the Se cycling, flux and balance in geo-ecological systems and to determine its effect on certain geographically distributed human health issues and animal health and nutrition.

In order to achieve the aims, a detail study was conducted with the following specific objectives:

(I) To determine Se level and other biochemically important trace element levels in water, soil, etc. in relation to geo-environment perspectives

(II) To estimate biologically labile Se fractionations in erythrocytes by glutathione peroxidase (GSH-Px) activity

(III) To determine Se migration pathways among geosphere pedosphere and biosphere

The outcome of the study set a baseline for the Se status in Sri Lanka and will thereby enable investigations on association between the Se status and geographically distributed endemic diseases in the country.

Methodology

Selenium and other important trace elements in environmental samples (soil and water), food items (rice, milk and animal feeds), and biological samples (human hair, nail, and bovine blood) were investigated. Surface water (n=31), groundwater (n=787), soil (n=103), rice (n=247), milk (n=68), and animal feed (n=50) were collected from three climatic zones; Wet Zone (WZ), Intermediate Zone (IZ) and Dry Zone (DZ) of Sri Lanka. Samples of human hair (n=77) and nail (n=76) were collected out of 79 biopsy

proven CKDu patients who attended the Renal Care Clinic of the Girandurukotte Base Hospital. Se and other bio-important trace elements contents were analyzed using ICP-MS after microwave aided acid digestion.

Glutathione Peroxidase activity (GSH-Px) in whole blood of cattle (n=80) was determined based on the method developed by Pagila and Valentine (1967) to assess the nutritional status of farm animals.

Results and Discussion

Surface Water

The levels of Se in surface water are below the maximum permissible levels set by both USEPA and WHO, However relatively high levels of Se, As, Cr, Mn, Fe, Co, Ni, Cu and Zn were found in upstream tributaries of the Mahaweli River, possibly originated from phosphate and organic fertilizers that are heavily applied in tea and vegetable cultivations. Despite the fact that water quality is still within the recommended levels, the results clearly indicated that the water quality of the tributaries and other surface water resources in the upper Mahaweli catchment are affected by trace metals. The evidences showed that anthropogenic impacts on the river were greatest in the upper part of the catchment, where intensive agricultural activities are widespread.

Groundwater

The total Se concentrations in drinking water from WZ, IZ and DZ ranged from <0.06 to 0.07, <0.06 to 1.68 and <0.06 to 20.8 µg L^{-1} respectively, with mean values of 0.03± 0.01 µg L^{-1} (n=39), 0.17± 0.27 µg L^{-1} (n=152) and 0.79± 1.69 µg L^{-1} (n=596), respectively (figure 1). Approximately 88% of the total samples (n=796) contained Se levels <1.0 µg L^{-1}. Comparatively higher Se levels were recorded in wells in sedimentary aquifers with a mean of 2.0± 2.78 µg L^{-1} (n=131). Extremely high values were recorded in a few locations probably due to agricultural contamination since there were minimal other natural or anthropogenic sources in those specific locations.

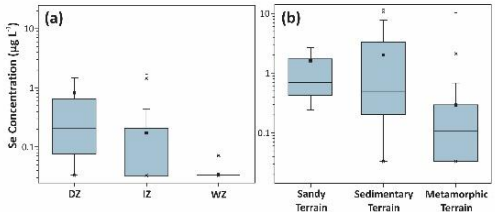

Figure 1. (a) Groundwater Se level variation according to the climatic zone and (b) according to the geological setting of the sampled region

Soil

Total Se concentration in the paddy soils from the IZ region varied from 0.02 to 1.05 mg kg^{-1} with a mean value of 0.41± 0.34 mg kg^{-1}; (n=18), while it varied from 0.01 to 0.99 mg kg^{-1} with mean value of 0.41± 0.23 mg kg^{-1}; (n=45) in the DZ. Higher Se contents were observed in natural forest soils and this may possibly due to the relationship of Se with organic matter content of soils.

Rice

Improved rice varieties from WZ, IZ and DZ showed the mean Se contents of 30± 33 µg kg^{-1} (n=81), 24± 29 µg kg^{-1}(n=70), and 44± 55 µg kg^{-1} (n=75), respectively implying lower dietary intake of Se through rice (table 1). However, significantly higher mean Se level (69± 64 µg kg^{-1} (n=21)) was recorded in traditional rice varieties and can be considered as a good nutritional source. Multivariate analysis revealed that As content in rice did not differ significantly among climatic zones (p=0.075) and among rice types (white and red varieties) (p=0.709) while Se differed among rice types (p=0.006). However, Cd content in rice differs drastically according to rice types (p=0.038) and climate zones (p= <0.001). However the reported values are less than the maximum permissible level for rice grain (table 1).

Table 1. Daily intakes of Se, Cd and As from improved and native rice

Category	Mean (µg/kg)			Daily Intake (µg)		
	Se	Cd	As	Se	Cd	As
WZ	30	128	48	8.5	36.3	13.9
IZ	24	54	38	6.8	15.3	10.9
DZ	44	68	41	12.5	19.2	11.8
Native rice	69	33	74	19.5	9.47	21.2
Sri Lanka	33	85	43	9.31	24.1	12.2

Daily rice consumption was considered as 0.284 kg (after Fordyce *et al.*, 2000) to calculate the daily intake with mean values

Milk and Animal Feed

The mean elemental concentrations of Se, Cd, As and Cu in cow milk were 18.1± 12.8 µg L^{-1}, 1.45± 1.19 µg L^{-1}, 7.35± 6.81 µg L^{-1} and 71.7± 54.8 µg L^{-1} (n=68) respectively. The mean concentrations of these elements in forage were 0.22± 0.19 mg kg^{-1}, 0.07± 0.07 mg kg^{-1}, 0.05± 0.06 mg kg^{-1} and 9.21± 8.76 mg kg^{-1} (n=36) and in concentrate feed were 0.33± 0.30 mg kg^{-1}, 0.20± 0.23 mg kg^{-1}, 0.23± 0.17 mg kg^{-1} and 2.28± 1.69 mg kg^{-1}, (n=14) respectively. The As content of the milk was well below the maximum permissible level however, interestingly 15% of the samples had Pb exceeding the permissible limit of 20 µg L^{-1}.

Human Hair and Nail

The total Se content in hair and nail of kidney patients (ranged between 0.043 µg g^{-1} , to 0.513 µg g^{-1} and 0.037 µg g^{-1} to 4.10 µg g^{-1}) and controls (ranged between 0.031 µg g^{-1} to 1.15 µg g^{-1} and 0.042 µg g^{-1} to 2.19 µg g^{-1}) were not significantly different. However the values were comparatively lower than the values reported from other countries possibly indicating Se deficiency among Sri Lankan population. Cutaneous manifestations observed in CKDu patients were mainly due to kidney failures (figure 2). Chemical analyses of hair and nails indicated that patients were not exposed to toxic levels of As (hair: 0.037±0.029 µg g^{-1}; nail: 0.079±0.057 µg g^{-1}) or the other elements studied.

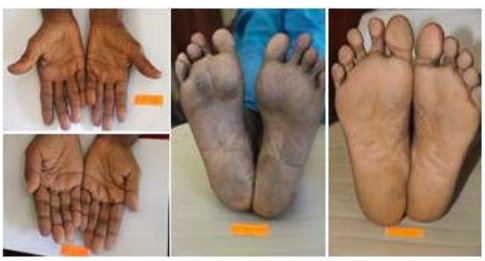

Figure 2. Examination for skin manifestations

Bovine Blood

GSH-Px activity of whole blood samples collected from different regions of the country varied from 320 to 2266 µkat L^{-1}. The activity of GSH-Px in erythrocytes indirectly confirms that considerable numbers of cattle suffered from insufficient Se levels.

Conclusion

Although the Sri Lankan soils contain adequate amounts of Se data reported herein indicate that the main staple food and drinking water did not provide sufficient amounts of Se. Se content signifies a decreasing pattern along the food chain, which imply that the substantial proportions of Sri Lankan population may be Se deficient irrespective of gender, age and occupational exposure. In addition, this study did not identify a relationship between CKDu and Se.

Source of Funding

This research work was financially supported by the Nation Research Council (NRC), Sri Lanka (Grant 12-102).

Selected References

Fordyce, F. M., Johnson, C. C., Navaratna, U. R., Appleton, J. D. and Dissanayake, C. B. (2000). Selenium and iodine in soil, rice and drinking water in relation to endemic goitre in Sri Lanka. *Science of the Total Environment*, 263, 127-141.

Jayatilake, N., Mendis, S., Maheepala, P. and Mehta, F.R. (2013). Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. *BMC Nephrology*, 14, 180.

Publications

Diyabalanage, S., Abekoon, S., Watanabe, I., Watai, C., Ono, Y., Wijesekara, S., Guruge, K. S. and Chandrajith, R. (2015). Has irrigated water from Mahaweli River contributed to the kidney disease of uncertain etiology in the dry zone of Sri Lanka? *Environmental Geochemistry and Health*, 38, 679-690.

Chandrajith, R., Diyabalanage, S., Premathilake, M., Hanke, C., von Gelden, R. and Barth, J. A. C. (2016). Controls of evaporative irrigation return flows in comparison to seawater intrusion in coastal karstic aquifers in northern Sri Lanka: Evidence from solutes and stable isotopes. *Science of the Total Environment*, 548, 421-428.

Diyabalanage, S., Navarathna, T., Abeysundara, H. T. K., Rajapakse, S. and Chandrajith, R. (2016), Trace elements in native and improved paddy rice from different climatic regions of Sri Lanka: implications for public health. *Springer Plus*, 5, 1864.

Diyabalanage, S., Fonseka, S., Dasanayake, D. M. S. N. B. and Chandrajith, R. (2017). Environmental exposures of trace elements assessed using keratinized matrices from patients with chronic kidney diseases of

uncertain etiology (CKDu) in Sri Lanka. *Journal of Trace Elements in Medicine and Biology*, 39, 62-70.

Diyabalanage, S., Dangolla, A., Mallawa, C., Rajapakse, S and Chandrajith, R. (2020). Bioavailability of selenium in cattle population in Sri Lanka based on qualitative determination of Glutathione Peroxidase (GSH-Px) activities. *Environmental Geochemistry and Health*, https://doi.org/10.1007/s10653-019-00395-3.

Diyabalanage, S., Kalpage, M. D., Mohotti, D. G., Dissanayake, C. K., Fernando, R., Frew, R. D. and Chandrajith, R. (2020). Comprehensive assessment of essential and potentially toxic trace elements in bovine milk and their feeds in different agro-climatic zones of Sri Lanka. *Biological Trace Element Research*.

Thesis Reference

Diyabalanage, R.S. (2019). Chemico-geography of Selenium and Its Impact on Food Chain Quality and Animal Health in Sri Lanka. Ph.D. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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MOLECULAR IDENTIFICATION AND DRUG SUSCEPTIBILITY OF NON-TUBERCULOUS MYCOBACTERIA

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Tuberculosis (TB) is an infectious disease caused by strains of *Mycobacterium tuberculosis* complex (MTC), which is currently one of the leading causes of death in the world (Moon *et al*. 2015). On the other hand, pulmonary infections caused by Non-tuberculous Mycobacteria (NTM), *Mycobacterium* species that are not members of MTC are also increasing rapidly (Gupta, *et al*. 2010).

As conventional detection methods such as, biochemical tests consume time (Gupta, et al. 2010), at present DNA amplification using Polymerase Chain Reaction (PCR) has allowed great progress in the rapid and accurate diagnosis of infections and new species of Mycobacteria. Application of SYBR green mediated real time PCR assay in clinical microbiology improves the diagnostics due to the increased specificity and the ability to detect two or more organisms in a single reaction (Richardson, et al. 2009). Rapid availability of the results is an added advantage of SYBR green mediated real time PCR assay over conventional PCR. Rapid identification will assist in avoiding unnecessary drug exposure and appropriate respiratory isolation. Thus, this study was conducted with following objectives:

(I) To identify the *Mycobacterium* species present in Bronchial washings using SYBR Green mediated multiplex, real time PCR

(II) To determine the drug sensitivity patterns of mycobacterial isolates

Methodology

Sputum smear negative bronchoscopy specimens (n = 150) were collected for a period of one year, from patients attending the General Hospital Kandy, Sri Lanka. The specimens were processed with modified Petroff's method and were cultured on Löwenstein– Jensen medium. DNA, extracted from the mycobacterial isolates were subjected to a SYBR green mediated real time multiplex, PCR assay with primers specific for the *M. Tuberculosis* complex, *M. avium* complex, *M. chelonae-M.abscessus* group and *M. fortuitum* group. DNA sequencing was performed for the species confirmation, by targeting the 16S rRNA gene and the drug susceptibility testing was performed for the molecularly identified isolates of *M. tuberculosis* and NTM.

Results and Discussion

The optimized SYBR Green mediated multiplex real-time PCR assay was able to identify the presence of genus *Mycobacterium* in 25 out of 26 AFB positive isolates, two *M. tuberculosis* complex, three *M. avium* complex and two isolates belonging to *M. chelonae-M. abscessus* group. DNA sequencing confirmed the presence of *M. tuberculosis*, *M. chelonae-M. abscessus, M. intracellulare, M. avium, Rhodococcus* sp. and *M. celatum*. Remaining isolates were identified as *Mycobacterium* sp. All the NTM isolates were sensitive to amikacin and seven were resistant to ciproflaxacin. Twenty-two of the NTM isolates and the isolate *Rhodococcus* was resistant to clarithromycin. The two isolates of *M. tuberculosis* were sensitive to all first line anti tuberculosis drugs.

Conclusion

The optimized SYBR Green mediated multiplex real time PCR assay could be an effective tool for the rapid differentiation of pathogenic *M. tuberculosis* complex from the opportunistic nontuberculous Mycobacteria and also it confirmed the presence of NTM in 15.3 % of the study population.

Source of Funding

Ministry of Science, Technology and Research, Sri Lanka.

Selected References

Moon, S. H., Kim, E. J., Tomono, J., Miyamoto, S., Mitarai, S., Kim, D. W. and Seki, M. (2015). Detection of Mycobacterium tuberculosis complex in sputum specimens using a loop-mediated isothermal amplification (LAMP) assay in Korea. *J Med Microbiol*.

Gupta, S., Bandyopadhyay, D., Paine, S. K., Gupta, S., Banerjee, S., Bhattacharya, S., Gachhui, R. and Bhattacharya, B. (2010). Rapid identification of mycobacterium species with the aid of multiplex polymerase chain reaction (PCR) from clinical isolates. *Open Microbiol J*, 4, 93-97.

Richardson, E., Samson, D. and Banaei, N. (2009). Rapid identification of Mycobacterium tuberculosis and nontuberculous mycobacteria by multiplex, real-time PCR. *Journal of Clinical Microbiology*, 47(5): 1497-1502.

Publications

Keerthirathne, T. P., Magana-Arachchi, D. N., Madegedara, D., and Sooriyapathirana, S. S. (2016). Real time PCR for the rapid identification and drug susceptibility of Mycobacteria present in Bronchial washings. *BMC Infectious Diseases*, 16(1), 607.

Keerthirathne, T. P., Weerasekera, D. K., Magana-Arachchi, D. N., and Dissanayake, N. L. A. (2016). Molecular diagnostics in a tuberculous patient with clinical nonresponse to standard treatment. *Sri Lankan Journal of Infectious Diseases*, 6(2).

Thesis Reference

Keerthirathne, T.P. (2018). Molecular Identification and Drug Susceptibility of Non-tuberculous Mycobacteria. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

Institutions where research was carried out

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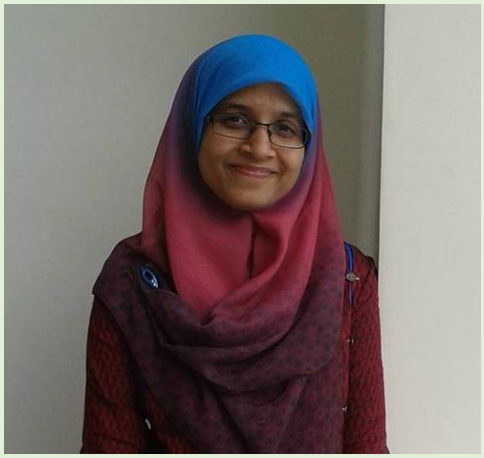
INTERACTION OF GLUCOSE OXIDASE (GOX) WITH NON-GLUCOSE SUGARS: ASSOCIATED CLINICAL IMPLICATIONS AND ITS APPLICATION IN ASSESSING α-AMYLASE ACTIVITY



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The glucose assay kit available in the market is comprised of the enzymes glucose oxidase (GOx) and peroxidase. Glucose oxidase is involved in the aerobic oxidation of β-D-glucose to glucono-δ-lactone with the concomitant reduction of molecular oxygen to hydrogen peroxide (figure 1). According to reported literature, GOx is highly specific for glucose and there is no significant reaction with other mono or disaccharides. However, from our preliminary studies the enzyme was found to interact with non-glucose sugars. Thus, the primary aim of the study was to determine the clinical implications associated with the interaction of GOx with other sugars and to develop a novel method to assess α-amylase activity based on the interaction of GOx with maltose.

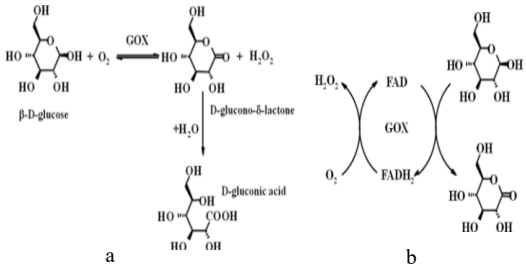

Figure 1. (a) Reaction catalyzed by GOx (glucose oxidase) and (b) GOx - mechanism of action

Main objectives:

(I) To develop a simple and cost-effective, laboratory method to measure α-amylase activity in a short period of time

(II) To determine the interaction of GOx sugars other than glucose and its clinical implications

Methodology

Development of a novel method to assess α-amylase activity using the GOx/POD kit

A simple, rapid and high-throughput analytical method for the detection and quantification of α-amylase inhibitory activity was developed using the glucose assay kit. The test was done in microtitre plates with a total volume of 260 µL

and an assay time of 40 minutes including the pre-incubation steps. The new method was tested for linearity, sensitivity, precision, reproducibility and applicability. Comparisons were made with the most commonly used 3,5-Dinitrosalicylic Acid (DNSA) method for determining α-amylase activity.

Determination of the interaction of GOx with other sugars and its clinical implications

Eleven sugars and sugar alcohols namely, maltose, galactose, fructose, mannose, xylose, arabinose, sucrose, lactose, sorbitol, mannitol, arabitol and xylitol were tested for its interaction with GOx. Initially, from each sample, 2 M solution was prepared and the interaction was tested by adding 100 µL of GOx/POD reagent in to 40 µL of sugar solution and 120 µL PBS. The content was allowed to stand for 15 min for the reaction to occur. Finally, the absorbance was measured.

Results and Discussion

Development of a novel method to assess α-amylase activity using the GOx/POD kit

Maltose is one of the main products of α-amylase action. Based on the interaction of GOx with maltose, a sound, simple, and high-throughput analytical technique was developed to determine α-amylase activity using the glucose assay kit. This method is based on the reaction of maltose with glucose oxidase (GOD) and the development of a red quinone (figure 2). According to the results, the newly developed GOx method demonstrated good accuracy, precision and reproducibility in determining α-amylase activity. GOx was successfully used to determine α-amylase and α-amylase inhibitory activity of the tested samples. HPLC studies confirmed the absence of glucose in the maltose standards thereby confirming the interaction of maltose with GOx. Furthermore, the hydrolysis products of α-amylase were also confirmed to interact with GOx.

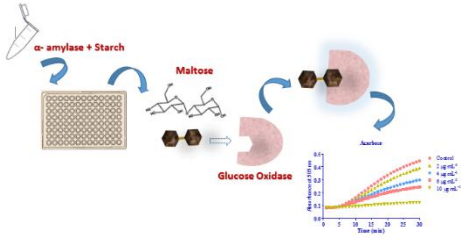

Figure 2. Schematic representation on the basic principle of the GOx method.

Determination of the interaction of GOx with other sugars and its clinical implications

GOx is one of the most widely used and highly recommended enzymes in glucose monitoring systems. According to the results, other than glucose, maltose, mannose, galactose, xylose, fructose and sorbitol acted as substrates for GOx and among these, the interaction with mannose, galactose, maltose, and xylose can be considered clinically significant. The affinity of GOx towards glucose was approximately 25-fold higher than that towards other sugar substrates (≥51 mM). Among the tested sugars, only maltose, D-galactose, D-xylose and D-mannose showed interaction at the concentration of 20 mM and no change was observed for sorbitol and D-fructose (figure 3). The positive interference caused by maltose and galactose in blood glucose reading was less than 3.75 and 2.2%, respectively. The presence of 20 mM of mannose, galactose, maltose and xylose raised the glucose concentration by 1.44±0.37 mM, 0.75±0.28 mM, 0.44±0.20 mM and 0.34±0.16 mM, respectively (average of all three kits).

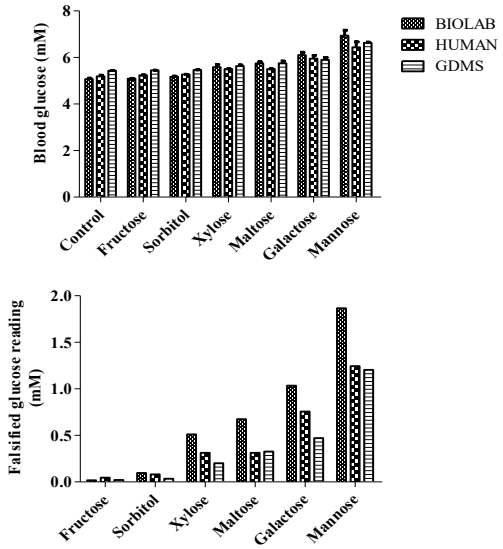

Figure 3. Interaction pattern of non-glucose sugars (20 mM) with three different glucose oxidase/peroxidase kits (a). Total blood glucose reading, (b). Falsified glucose reading.

Conclusion

Since, maltose level in blood following infusion of maltose-containing agents and galactose level in galactosaemic patients are not elevated to a degree to alter the meter readings, the specificity of GOx based glucose monitoring systems can be considered sufficient in most clinical situations. However, the positive interference from mannose was above 10%. Though there are no reports regarding mannose interference in blood glucose reading, this interaction should be taken into careful consideration as mannose is used in some intravenous therapies. In summary, despite its interaction with non-glucose sugars, GOx based systems are safe for blood glucose monitoring since the blood concentrations of these sugars after a treatment regime are not elevated to a level that can cause significant interference.

Source of Funding

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Selected References

Bankar, S. B., Bule, M. V., Singhal, R. S. and Ananthanarayan, L. (2009). Glucose oxidase - An overview. *Biotechnology Advances*, *27*(4), 489–501.

Courjean, O. and Mano, N. (2011). Recombinant glucose oxidase from Penicillium amagasakiense for efficient bioelectrochemical applications in physiological conditions. *Journal of Biotechnology*, *151*(1), 122–129.

Disse, E., and Thivolet, C. (2004). Hypoglycemic coma in a diabetic patient on peritoneal dialysis due to interference of icodextrin metabolites with capillary blood glucose measurements. *Diabetes Care*, *27*(9), 2279.

Publications

Visvanathan, R., Qader, M.M., Jayathilake, C., Liyanage, R. and Sivakanesan, R. (2020). Critical review on conventional spectroscopic α-amylase activity detection methods: merits, demerits, and future prospects. *Journal of the Science of Food and Agriculture,* 100, 2836-2847.

Visvanathan, R., Jayathilake, C., Liyanage, R. and Sivakanesan, R. (2019). Applicability and reliability of the glucose oxidase method (GOD) in assessing α-amylase activity. *Food Chemistry,* 275, 265-272.

Visvanathan, R., Jayathilake, C. and Liyanage, R. (2016). A simple microplate-based method for the determination of α-amylase activity using the glucose assay kit (GOD method). *Food Chemistry,* 211, 853–859.

Thesis Reference

Visvanathan, R. (2018). Interaction of Glucose Oxidase with Non-glucose Sugars: Associated Clinical Implications and Its Application in Assessing α-amylase Activity. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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ORIGIN OF THERMAL SPRINGS AT THE HIGHLAND/ VIJAYAN TECTONIC BOUNDARY IN SRI LANKA: A COMBINED GEOPHYSICAL AND PETROLOGICAL STUDY

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Sri Lanka has nine hot springs, categorized as low enthalpy geothermal systems, according to the surface outflow temperatures (35 – 72 °C), and calculated heat source temperature (140-160 °C). Two main hypotheses have been proposed by previous researchers on the origin of hot springs, namely, serpentinite-like mineral-based heat sources located in the Highland Complex and Vijayan Complex (HC/VC) boundary zone and dolerite intrusions as a heat source and deep percolating groundwater acquiring heat from the natural geotherm. However, none of the above hypotheses have been confirmed yet.

The study was carried out with following objectives:

(I) To find the origin of the hot springs and its relationship with the host rocks

(II) To understand the geo-petrochemical nature of HC/VC boundary

Methodology

Mahapelessa and Nelumwewa hot spring fields were selected as two study sites, based on the proximity to the HC/VC boundary (figure 1). A novel approach was attempted in this study, by integrating geological, geophysical, petrological, and whole-rock geochemical study methods. Geophysical methods include Magnetotelluric (MT) and Time-domain electromagnetic (TDEM), Two-dimensional electrical resistivity, and Magnetic method. Basic geological mapping, including lithological and structural mapping, were completed covering the study area. ICP-MS and XRF analyses were conducted for selected samples to study the geochemistry of the basement rocks in the study area in proximity to the HC/VC boundary in Sri Lanka.

Results and Discussion

Petrological and geochemical results from this study suggest a subduction-related volcanic arc and within-plate tectonic origin for the rocks in the VC and HC/VC boundary zone. Therefore, cooling magma bodies, in the deep crust in both hot spring fields can be interpreted as magmatic intrusions that originated as a result of subduction setting associated with rifting at post-Gondwana times (150-200 Ma).

In Nelumwewa hot spring field negative magnetic anomalous zones are identified parallel to the direction of the regional shear zone (figure 2). These low magnetic zones are interpreted as geothermal flow paths along with the fractures in the shear zone. Very low resistivity zone (1-10 Ωm) was identified in 16 - 12 km depth in the Nelumwewa resistivity profile interpreted as a cooling magma body. This magma intrusion is interpreted as the heat source of the Nelumwewa hot spring field. Low resistive zones identified in 8 - 4 km depths which are of 3 km wide in the Nelumwewa resistivity profile is interpreted as the geothermal water accumulation zones. Another low resistive zone occurs in the profile which is close to the surface (<2 km) in Nelumwewa hot spring field, is interpreted as shear zone-controlled mineralization and geothermal flow paths with local small-scale geothermal accumulation zones.

Figure 1. Geological map of Sri Lanka (after Cooray, 1994), showing seven major hot springs

In Mahapelessa hot spring field a shear zone is observed, starting from the HC/VC boundary and extending to the hot spring filed. Very low resistivity zone (1-10 Ωm), identified in 18-12 km depth, interpreted as cooling magma body which is possibly the heat source of the hot spring. Low resistivity zone (1-100 Ωm) is observed starting from 6 km depth and extending to the surface with apparently following the angle of the HC/VC boundary. This zone with three low resistive pockets is interpreted as, local geothermal accumulation zones and flow paths.

Low resistivity zone (1-100 Ωm) identified in the South-Western direction to the hot spring, located in 0.5-1.5 km depth is interpreted as serpentinite, magnetite like mineralization which is observed in the nearby terrains in the surface. Another low resistivity zone (100-500 Ωm), observed starting from the lower crust between HC and VC, is interpreted as the HC/VC mixed zone, which is possibly highly fractured due to the collisional tectonics. Therefore, these deep extending fractures in the HC/VC mixed zone, probably bring the heat from the mantle or deep crust to the surface.

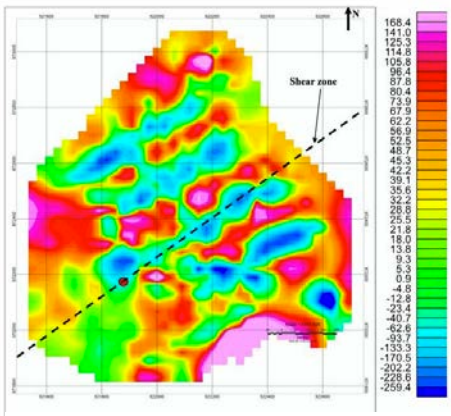

Figure 2. Dirunal and Base corrected magnetic anomaly map of the Nelumwewa hot spring field. Small red dot represents the location of the hot spring

Conclusion

From the results obtained, it can be concluded that heat sources for both hot springs studied are located at the closest point to the surface below the 12 km depth, which is intruded along the fractures formed in the subduction collision between HC/VC. The heat from the source is transferred to the upper crust and the surface along with a similar fracture system. Hot water flow paths appear to start from the crustal geothermal accumulation zones (6-2 km) in the upper crust in both hot spring fields.

Shallow geothermal flow paths and directions are mainly controlled by the local fracture systems, especially by the shear zones identified in both Mahapelessa and Nelumwewa Hot springs. Deep resistivity structure profiles modeled from the Magnetotelluric (MT) method reveals, very low resistivity zones (1 -10 Ωm) located in 12 km depth and extends deeper, in both hot spring fields.

It can also be suggested that as still-hot magma bodies may be present beneath the region, due to the heat transferring from the mantle or lower crust along the fractures formed due to subduction tectonics.

Source of Funding
National Institute of Fundamental Studies, Kandy, Sri Lanka. National Science Foundation funding for travel and accommodation during the geochemical analysis period in Japan, is highly appreciated.

Selected References

Cooray, P. (1994). The Precambrian of Sri Lanka: a historical review. *Precambrian Research,* 66(1-4), 3-18.

Dahanayake, K., and Jayasena, H.A.H. (1983). Textural studies of some granites and gneisses from the Precambrian Vijayan complex of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka,* 11(1), 77–86.

Publications

Bandara, H.M.D.A.H., Sooriyarachchi, N.B., Subasinghe, N.D. (2019). Near-surface resistivity and geostructural study of the Mahapelessahot spring field, *SRI LANKA. Journal of Geological Society of Sri Lanka*, 20-1, p.11-16.

Bandara, H.M.D.A.H. Sooriyarachchi, N.B. Dissanayake, C.B. Subasinghe, N.D. (2019). Geothermal Energy - Potential applications in Sri Lanka. *Journal of Vidulka National Energy Symposium*, 5(1), p.161-173.

Thesis Reference

Bandara, H.M.D.A.H. (2020). The Origin of the Thermal Springs at the Highland/Vijayan Tectonic Boundary in Sri Lanka: A Combined Geophysical and Petrological Study. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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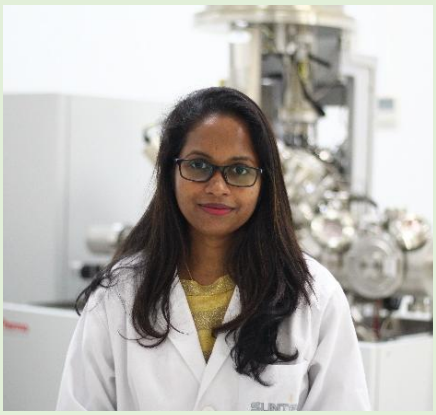
METHODS FOR ENHANCING SOLUBILITY OF RESVERATROL



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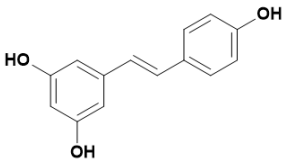
A considerable number of nutraceutical and pharmaceutical compounds have shown low solubility and bioavailability. Poor solubility of pharmaceuticals is a serious problem, since solubility affects the bioavailability and efficacy. Over 80% of drugs are solid dosage forms mainly as tablets. About 40% of marketed drugs are reported to have low solubility (Babu and Nangia 2011). Resveratrol (3,5,4′-trihydroxystilbene) is a bioactive polyphenol found in various fruits and vegetables including red grape skin, peanuts, mulberry, cranberry, blueberry, and jackfruit (Billes et al. 2007). The trans-isomer of resveratrol (RES) (figure 1) is the most stable form due to steric arrangement of substituents (Trela and Waterhouse, 1996).



trans-resveratrol

Figure 1: Chemical structure of *trans*-resveratrol

The bioactive herbal ingredient RES is known for its multiple pharmacological activities such as antioxidant (Lastra & Villegas, 2007), anticancer (Jang et al., 2016), cardioprotective, anti-inflammatory, anti-aging and neuro-protective activity. But therapeutic applications of RES are limited due to its low water solubility and poor bioavailability (Robinson, Mock, and Liang 2015). Therefore, more suitable strategies are needed to achieve better clinical effectiveness of RES (Zupančič, Lavrič, and Kristl 2015).

The study was carried out with the following objectives:

(I) To synthesize cocrystals and eutectics of resveratrol with ferulic acid (FA) by different methods to enhance the solubility and antioxidant activity of resveratrol

(II) To synthesize esters of resveratrol with *trans*-cinnamic acid (CIN)

(III) To determine the antioxidant, antimicrobial activities of synthesized esters

(IV) To determine the hydrolysis of synthesized esters in artificial sweat

Methodology

Synthesis of cocrystals and eutectics

Synthesis of cocrystals of RES: FA was carried out by neat grinding (NG), liquid-assisted grinding (LAG), slow evaporation (SE), solvent diffusion (SDif) and reflux methods. Ethanol was used as the solvent for the LAG, SE, SDif and reflux methods. Ethanol, methanol, acetonitrile and hexane were used for SE and SDif methods either singly or as solvent combinations. Different stoichiometric molar ratios of RES and FA were used to screen for cocrystal formation by Differential Scanning Calorimetry (DSC). In the preliminary screening, 1:1, 1:2, 1:3, 2:1 and 3:1 stoichiometric molar ratio of RES and FA were used to synthesize the cocrystals.

Synthesis of eutectics of RES: FA was carried out by liquid-assisted grinding (LAG) and spray drying (SD) methods.

LAG - Definite stoichiometric molar ratios of RES and FA (1:1, 1:2, 1:3, 2:1 and 3:1) were ground for 30 min, with a catalytic amount of ethanol, using mortar and pestle.

SD - Definite stoichiometric molar ratios of RES and FA were dissolved individually in minimum amount of ethanol. FA solution was added drop wise to RES solution while stirring. The resultant solution was spray dried using a modified spray dryer developed from an airbrush. The solution was delivered at 1 - 2 bar pressure with inert Argon gas to a heated (120 °C) nonstick surface. Resultant powder mixtures were collected and slightly ground to obtain clog free fine powder.

Saturation solubility in water and antioxidant activity of eutectic mixtures were determined.

Synthesis of esters

Synthesis of conjugates of RES and CIN was carried out by DCC/ DMAP coupling reaction in anhydrous tetrahydrofuran (THF) using modified Steglich esterification at 0 °C in the dark. Formation of resveratrol esters was confirmed by Thin Layer Chromatography (TLC) and direct Mass Spectral (MS) analysis.

Results and Discussion

Single crystal XRD results of single crystals obtained from RES and FA experiments suggested that the single components were crystallizing out and no formation of cocrystals of RES and FA occurred. The formation of eutectics was confirmed by the binary phase diagrams supported by the single lower melting endotherm of the Differential Scanning Calorimetry (DSC) thermograms with comparatively no significant changes compared to the starting materials. Powder X-ray diffraction (PXRD) and Fourier-transform infrared spectroscopy (FTIR) confirmed this. Therefore, the mixtures synthesized by NG and LAG were screened for the eutectic formation. Further RSR and SD methods were carried out to synthesize eutectics. Eutectics of RES with FA were obtained by NG, LAG and SD methods in molar ratios of RES to FA in 1:1, 1:2 and 1:3 compositions whereas the RSR method resulted in the eutectic composition of RES: FA in 1:1 combination only. The eutectics of RES with FA have not been reported previously.

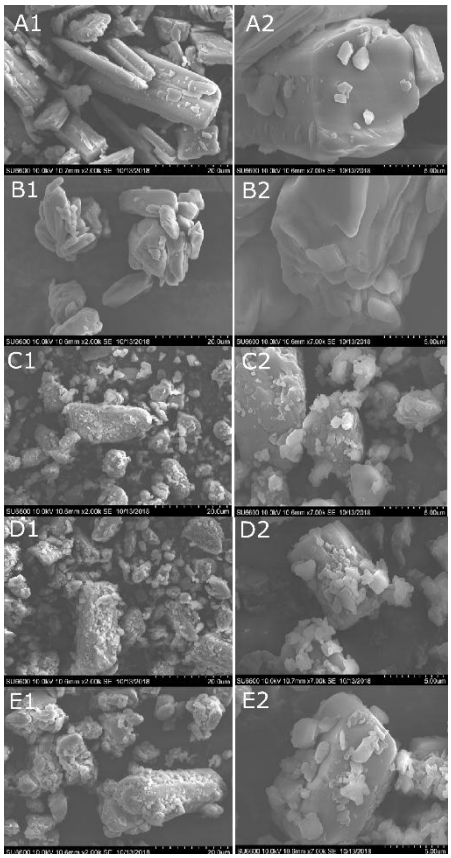


Figure 2: SEM images of A1: RES (2k), A2:RES (7k), B1:FA (2k), B2: FA (7k), eutectics of RES:FA by LAG; C1- 1:1 (2k), C2- 1:1 (7k), D1- 1:2 (2k), D2- 1:2 (7k), E1-1:3 (2k), E2- 1:3(7k)

The aqueous solubility of eutectics

The saturation solubility of RES in water increased in the eutectics synthesized by LAG method, whereas the saturation solubility of RES in the eutectics synthesized by SD was lower than RES alone. In the SD method, both RES and FA are in ethanol solution and on rapid evaporation of the solvent, the coprecipitating solid could be in a metastable state. During coprecipitation of the metastable state from solution, the intermolecular forces could be differently formed lowering the solubility. The particle sizes of the RES in eutectics in SD method are larger than resveratrol alone due to aggregation or agglomeration of RES particles (Figure 2). This may have led to lowered solubility of RES in eutectics prepared by SD method. In eutectics prepared by the LAG method, the particle size of the RES particles is smaller than free RES. Since smaller RES particles are covered by more soluble FA particles, as FA dissolves faster, leading to RES dissolving more. Solubility of RES is enhanced by the formation of eutectics with FA by LAG method.

The antioxidant activity of eutectics

The antioxidant activity of the eutectics measured by the DPPH assay indicated a 25-40% increase in antioxidant activity for the three eutectic compositions synthesized by both LAG and SD methods. Antioxidant activity of eutectics synthesized by SD method has increased up to 39%, while the antioxidant activity of eutectics synthesized by LAG method was 34%. The antioxidant activity of FA is higher than RES by 10%, whereas 2-3 time increase in the antioxidant activity was found in the eutectics. Although the eutectic mixtures are not showing true synergism, some synergism can be seen in the antioxidant activity in the eutectics synthesized by LAG and SD. The antioxidant activity is higher when RES eutectics of FA rather using RES alone.

Esters of resveratrol

Resveratrol tricinnamate was synthesized by Steglich esterification. The triester was obtained in 27% yield with low reaction time. Use of THF in Steglich reaction minimizes the use of chlorinated solvents in the reaction. Synthesized molecule did not show antioxidant activity due to the lack of phenolic groups. Therefore, it is evident that the phenolic OH groups are required for the antioxidant activity of RES and RES based derivatives.

The synthesized resveratrol tricinnamate lacked the antimicrobial activity against *Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*, while both RES and CIN showed antimicrobial activity towards *Bacillus cereus* and *Pseudomonas aeruginosa.* The lack of carboxylic groups and phenolic OH groups may have contributed toward low antimicrobial activity of resveratrol tricinnamate.

Conclusion

The solubility of RES is enhanced by formation of eutectics with FA by LAG method. The antioxidant activity is higher when using RES eutectics of FA rather using RES alone. Synthesized resveratrol tricinnamate conjugate did not show antioxidant activity. Limited hydrolysis of the resveratrol tricinnamate in artificial sweat suggests the possible use of resveratrol tricinnamate as a prodrug in topical formulations.

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NIMS Internship -National Institute of Material Science (NIMS), Tsukuba, Japan.

Selected References

Babu, N. J. and Nangia, A. (2011). Solubility Advantage of Amorphous Drugs and Pharmaceutical Cocrystals. *Crystal Growth & Design*, 11(7), 2662–79.

Billes, F., Mohammed-Ziegler, I., Mikosch, H. and Tyihák, E. (2007). Vibrational spectroscopy of resveratrol. Spectrochimica Acta - Part A: *Molecular and Biomolecular Spectroscopy,* 68(3), 669–79.

Trela, B. C. and Waterhouse, A. L. (1996). Resveratrol: Isomeric molar absorptivities and stability. *Journal of Agricultural and Food Chemistry,* 44(5), 1253–7.

Lastra, C. A. de la. and Villegas, I.(2007). Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. *Biochemical Society Transactions,* 35(5), 1156–60.

Robinson, K., Mock, C. and Liang, D. (2015). Pre-formulation studies of resveratrol. *Drug Development and Industrial Pharmacy,* 41(9), 1464–9.

Zupančič, Š., Lavrič, Z. and Kristl, J. (2015). Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature. *European Journal of Pharmaceutics and Biopharmaceutics*, 93,196–204.

Publications

Buddhika, R. B. J., Priyadarshana, G., Welideniya, D., Karunaratne, D. N. and Karunaratne, V. (2018). Eutectic compositions of Resveratrol for oral administration. *Asian Symposium on Medicinal Plants, Spices and Other Natural Products XVI*. 239.

Thesis Reference

Buddhika, R. B. J. (2019). Methods for Enhancing Solubility of Resveratrol. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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Japan

STUDY OF 2D MATERIALS USING ADVANCED ELECTRON MICROSCOPY

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Two dimensional (2D) materials have gained attention as a route to new technologies and electronics. Graphene was the first such material to be studied. It was followed by other 2D materials such as phosphorene and transition metal dichalcogenides (TMD) and the interest in these latter materials was due to their intrinsic band gap compared to the zero band gap of graphene. The interesting properties shown by 2D materials is due to their specific atomic arrangement. To understand the nature of these 2D materials, atomic scale studies through advanced electron microscopy is essential. Graphene, phosphorene and MoS_2 were selected as the 2D materials to be studied in this research work using a high resolution transmission electron microscope (HRTEM).

The study was carried out with following objectives:

(I) To optimize dose parameters development to electron beam sensitive 2D materials on HRTEM analysis, energy dispersive x-ray spectroscopy (EDX), and electron energy loss spectroscopy (EELS)

(II) To synthesize graphene oxide (GO) from Sri Lankan vein graphite using improved Hummers method and reduction of GO to obtain reduced graphene oxide (RGO)

(III) To synthesize 2D phosphorus and MoS_2 from bulk, using chemical exfoliation method in different organic solvents

(IV) To study phosphorene and single layered MoS_2 in atomic scale using HRTEM, SAED, EDX, EELS, and Raman spectroscopy

(V) To study the phase transition behavior of MoS_2 through HRTEM

Methodology

Sri Lankan vein graphite was exfoliated with Improved Hummers method. Black phosphorus and bulk MoS_2 were exfoliated in different organic solvents to obtain single to few atomic layers. Graphene, phosphorene, and single layered MoS_2 were characterized using advanced electron microscopic techniques.

Results and Discussion

Study of Graphene oxide and reduced graphene oxide from Graphite

Graphene oxide was prepared using improved Hummers method and chemically reduced to obtain reduced graphene oxide. Both GO and RGO were studied through HRTEM facilitated with EELS. Inter atomic layer distance for few-layered RGO was measured using HRTEM. Selected area electron diffraction pattern (SAED) was acquired and the characteristic intensity profile for single layered graphene was obtained along with for few layered graphene. The EELS spectrum shown in figure 1 clearly displays the appearance of a peak in between 1s to π* and σ* transitions due to the oxygen functionalities in GO than RGO. Moreover, the π* peak has been lowered in GO than RGO, while a new peak around 290 eV appeared due to the presence of high oxygen content in GO. This peak might be mainly due to the oxygen atoms bonded as C=O, and COOH as reported by A. Ganguly *et. al.* (2011). The characteristic energy loss near edge structures confirms the different oxygen levels. Furthermore, EELS core loss and X-ray XPS studies confirmed the reduction of oxygen in RGO. The characteristic peaks in the Raman spectrum for GO and the peak behavior with increasing temperature were studied.

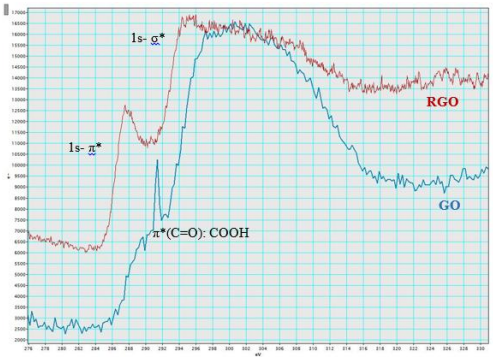

Figure 1. EELS Carbon K edge comparison on GO and RGO

Exfoliation of black phosphorus to obtain phosphorene

Single to few layered phosphorene was obtained from black phosphorus with the ultra-sonication assisted solvent exfoliation technique in N_2 environment with different organic solvents (DMSO, DMF, DMAC, and ethanol). The products were confirmed with HRTEM imaging and SAED patterns. Inter atomic layer distances for phosphorene in DMSO were obtained as 3.39 Å, and 2.22 Å which were closer to the theoretical atomic distances (figure 2). Prolong exposure to the electron beam showed the appearance of defects in the back-folded edge. The back-folded edge of phosphorene was obtained for two layers. The distance between two phosphorene layers was obtained as 5.1 Å which is very closer to the characteristic theoretical value. However, the puckered structure was not seen for the high resolution image and it appeared as straight lines. An aberration corrected HRTEM should be used to obtain a higher resolution. The electron diffraction values were correlated with theoretical atomic distance values. Furthermore, EELS study was done for both core loss and low loss regions of few layered phosphorene. HRTEM data confirmed that DMSO is the best solvent for the exfoliation. Furthermore, the Raman spectrum confirmed the few layered phosphorene structures.

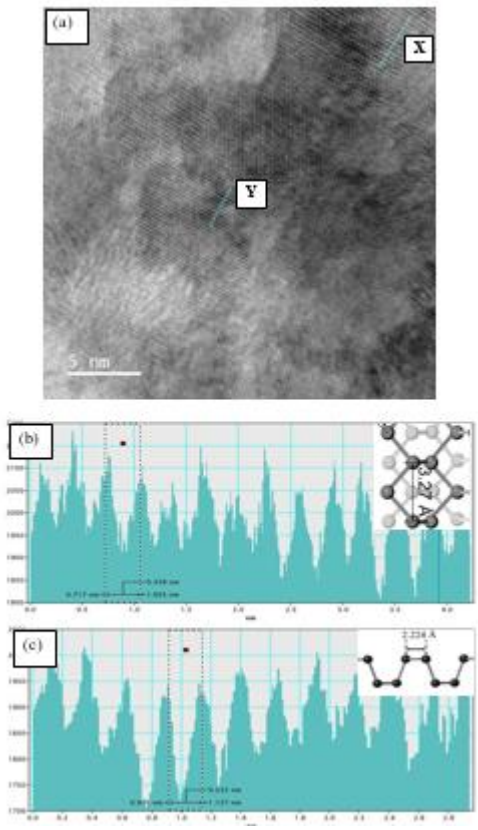

Figure 2. (a) HRTEM image of phosphorene exfoliated in DMSO (b, and c) Intensity profile

for position X, position Y, respectively with schematic diagram of atomic distance given as insets

Exfoliation of MoS_2 to obtain single layered MoS_2
The most popular TMD material, MoS_2 was selected for the atomic scale study. Solvent assisted exfoliation technique was followed. A phase transformation from 2H to 1T was observed from the characteristic atomic layer arrangement in back-folded edge. The surface atomic arrangement with characteristic honeycomb structure was observed for the first time from the facile synthesis route of exfoliation (figure 3). Characteristic Raman peaks confirmed the reduction of the number of layers in MoS_2.

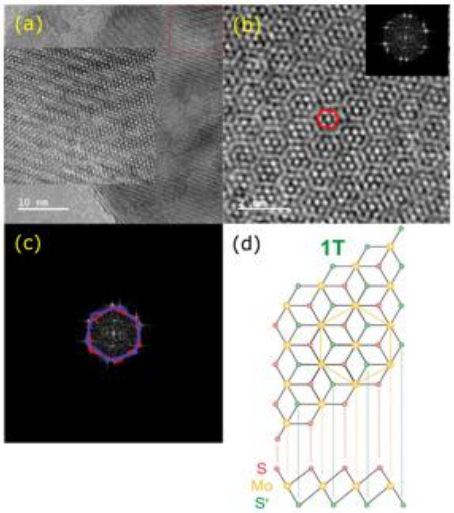

Figure 3. (a) HRTEM image of exfoliated MoS_2 with zoomed image in inset (b, c) processed image of the zoomed area (d) schematic diagram of 1T MoS_2 marked with hexagonal shape in the repetitive unit for the honeycomb like structure

Two micro analysis techniques namely, energy dispersive x-ray spectroscopy and wavelength dispersive x-ray spectroscopy (WDX) were reviewed for quantification of MoS_2 and compared with the EELS quantification with core loss higher energy loss range (figure 4). High energy core loss EELS can be recommended for the composition analysis of 2D materials. Chemical shift of Mo and S were studied through XPS.

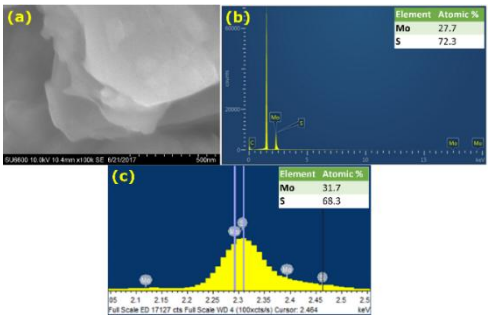

Figure 4. (a) SEM image of exfoliated MoS_2 (b) EDX spectrum of exfoliated MoS_2 (c) WDX spectrum of exfoliated MoS_2

Conclusion

Advanced electron microscopic study of Sri Lankan vein graphite is presented for the first time in this research work. The HRTEM images proves the few layered RGO sheets. The EELS carbon K edge and fine structures obtained for GO and RGO confirm the presence of lesser content of oxygen in RGO. The core loss edge of carbon change due to the presence of oxygen is remarkable with the limitations of our EELS system. Raman studies show the characteristic peaks for graphite, GO and RGO which matches well with literature data. The XPS analysis confirms less amount of oxygen present in RGO compared to GO. The advanced electron microscopic techniques together with other molecular spectroscopic analysis techniques allowed to obtain a comprehensive study of structural and chemical features of Sri Lankan vein graphene in the atomic scale.

Black phosphorus was exfoliated in DMSO, DMF, DMAC, and ethanol solvents in an inert nitrogen environment. The exfoliation was good in the sample in DMSO compared to other organic solvents which was confirmed with HRTEM imaging and further with diffraction data. HRTEM imaging and selected area diffraction pattern illustrates the few layered 2D phosphorus. The core loss edge and the plasmon range of the obtained EELS spectrum confirmed the presence of phosphorus. Raman study confirms the exfoliation of phosphorene.

MoS_2 monolayers and few layers were obtained by the ultra-sonication assisted exfoliation technique in ethanol medium. The characteristic distances between MoS_2 layers were observed using HRTEM. The bulk sample of MoS_2 was in the 2H phase, which was confirmed with XRD

and Raman spectroscopy. Furthermore, the distance between the characteristic A_{1g} and E^1_{2g} Raman peaks were reduced with the reduction in the number of layers in MoS_2. HRTEM analysis of the back-folded edge shows the 1T phase of MoS_2. Furthermore, the characteristic pattern of the honey comb-like arrangement of the MoS_2 surface due to the interference of Moire patterns in the 1T structure of MoS_2 was directly observed in HRTEM. Having compared the three analytical techniques, namely, EDX, WDX, and EELS, high energy core loss EELS can be recommended for the composition analysis of 2D materials. The ultrasonication-assisted solvent exfoliation technique is a facile route to obtain a few layer MoS_2, in which the back-folded edges can exhibit the 1T phase.

Selected References

Marcano, D. C., Kosynkin, D. V., Berlin, J. M., Sinitskii, A., Sun, Z., Slesarev, A., Alemany, L. B., Lu, W. and Tour, J. M. (2010). Improved Synthesis of Graphene Oxide. *ACS Nano,* 4(8), 4806-4814.

Ganguly, A., Sharma, S., Papakonstantinou, P. and Hamilton, J (2011). Probing the Thermal Deoxygenation of Graphene Oxide Using High-Resolution In Situ X-ray-Based Spectroscopies. *The Journal of Physical Chemistry C,* 11**5**(34), 17009-17019.

Publications

Dahanayake, D., Gunasekara, S., Jayaweera, V., Sandaruwan, C., Karunarathne, V. and Amaratunga, G. A. (2018). Atomic scale study for the structural transformation of single layered MoS2. *CrystEngComm*, 20(41), 6482-6489

Thesis Reference

Damayanthi, A.D.M. (2019). Study of 2D Materials Using Advance Electron Microscopy. Ph.D. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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CHEMISTRY AND BIOACTIVITY OF SECONDARY METABOLITES PRODUCED BY ENDOPHYTIC FUNGI IN THE FRUITS OF *PHYLLANTHUS ACIDUS* AND *ELAEOCARPUS SERRATUS*

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Chemical compounds produced by plants, animals, microorganisms and marine organisms play major role in a wide range of applications such as agriculture, medicine, pharmaceutical, cosmeceutical and food industry. Endophytic fungi are defined as living organisms which colonize internal tissues of higher plants without showing any external disease symptoms. These fungi spend a part or whole of their life cycle inside the host plant. Endophytes resulting from the recombination of host plant genes are more capable of producing the same vital and rare bioactive compounds produced by the host plant The aim of my study was to investigate the chemistry and bioactivity of endophytic fungi associated with Sri Lankan edible fruits, *Phyllanthus acidus* and *Elaeocarpus serratus* since these fruits are consider as rich sources of potassium, dietary fiber, vitamin C, and folic acid.

The study was carried out with following objectives:

(I) To isolate and identify fungi associated with the fruits of *Phyllanthus acidus* of family Phyllanthaceae and *Elaeocarpus serratus* of family Elaeocarpaceae

(II) To investigate chemistry and bioactivity of secondary metabolites produced by isolated endophytic fungi

Methodology

Isolation of endophytes

Mature fruits of *Phyllanthus acidus* and *Elaeocarpus serratus* were collected from home gardens. Fruits were surface sterilized by triple sterilization procedure. Small pieces were cut from the flesh of the fruits and placed on Potato Dextrose Agar (PDA) plates under aseptic conditions. PDA plates were incubated at room temperature for 3-7 days. Plates were observed every other day and fungi emerging from the pieces of fruits were sub cultured repeatedly until pure cultures of fungi were obtained.

Identification of endophytic fungi

The four endophytic fungi isolated from *P. acidus* and *E. serratus* were identified as *Daldinia eschscholtzii* & *Biscognauxia capnodes* from P.

acidus and *Neofusicoccum parvum* & *Neopestalotiopsis saprophytica* from *E. serratus* by morphologically as well as molecular methods at the Gene Tech Institute of Sri Lanka and University of Peradeniya, Sri Lanka.

Large scale culturing of endophytic fungi in PDB media

Potato Dexstrose Broth (PDB) was used as the culturing medium The flasks were inoculated with the respective fungus and incubated at room temperature for 21 days. After 10 days of undisturbed incubation, the flasks were shaken at 100 rpm on a laboratory shaker for 2 h per day until extraction. After 21 days, the medium was filtered and the filtrate was partitioned with EtOAc. The EtOAc extract of the culture broth was evaporated to dryness using a rotary evaporator. The mycelium of each fungus was crushed into small pieces and extracted into EtOAc followed by MeOH.

Biological activity studies

All crude extracts and isolated compounds were subjected to antifungal activity against *Cladosporium cladosporioides*, antioxidant activity against DPPH free radicals, cytotoxic activity against brine shrimp (*Artimia salina*), phytotoxic activity by seed germination inhibition of lettuce seed (*Lactuca sativa*) and enzyme inhibitory activity against α-amylase enzyme to determine biological activity of both crude extracts and compounds.

Isolation and identification of compounds

The TLC patterns of EtOAc extracts of culture broth and mycelium of each fungus were found to be almost similar. Therefore, the EtOAc extracts of culture broth and mycelium of respective fungus were combined. The four EtOAc extracts of the four fungi were separated by chromatographic techniques. The extracts were chromatographed over silica gel using *n*-hexane-EtOAc-MeOH followed by *n*-hexane- CH_2Cl_2-MeOH or CH_2Cl_2-MeOH, Reverse phase silica (RP-18) using water/MeOH and sephadex LH- 20 using MeOH followed by preparative thin layer chromatography to furnish 20 compounds.

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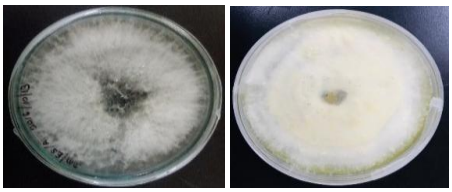
Results and Discussion

Chemistry and bioactivity of four endophytic fungi isolated from two Sri Lankan edible fruits were investigated in this study. Endophytic fungi, *D. eschscholtzii* and *B. capnodes* from the fruits of *P. acidus* and *N. parvum* and *N. saprophytica* from the fruits of *E. serratus* were isolated following standard methods and identified by morphologically and by molecular means (figure 1).

A B



C D

Figure 1: Endophytic fungi isolated from *P. acidus* (A&B) and *E. serratus* (B&C)
A: *Biscogniauxia capnodes*
B: *Daldinia eschscholtzii*
C: *Neofusicoccum parvum*
D: *Neopestalotiopsis saprophytica*

Pure cultures of each fungus were inoculated in to PDB liquid media. After fermentation medium was extracted with ethyl acetate (EtOAc) and the mycelium was sequentially extracted with EtOAc and methanol (MeOH). Crude EtOAc extract of *D. eschscholtzii*, *B. capnodes* and *N. parvum* displayed antifungal activity against *C. cladosporioides*, antioxidant activity against DPPH free radicals, cytotoxic activity against brine shrimp (*A. salina*), phytotoxic activity by seed germination inhibition of lettuce seed (*L. sativa*). EtOAc extract of *N. saprophytica* showed only weak antioxidant activity. None of the MeOH extracts displayed significant bioactivity for any bioassays given above. EtOAc extracts of each fungus subjected to chromatographic separations over column chromatography and PTLC to furnish total of 20 secondary metabolites. EtOAc extract of *D. eschscholtzii* yielded four compounds, 1-(2,6-dihydroxyphenyl)butan-1-one, 1,8-dimethoxy napthalene, 5-hydroxy-2-methylchromane-4-one and 8-methoxynaphthalen-1-ol; *B. capnodes* afforded seven compounds, 5-methylmellein, 8-methoxy-3,5-dimethylisochroman-1-one, 6,8-Dimethoxy-3-methylisocoumarin, 6-*O*-methylreticulol, (3*R*)-7-hydroxy-5-methylmellein,

reticulol and orcinol; *N. parvum* furnished six compounds, (*R*)-7-hydroxy mellein, (*R*)-5-hydroxymellein, (6*R*,7*S*)-dia-asperlin, (*R*)-(-)-mellein, (3*R*,4*R*)-4,7-dihydroxymellein and 13,14,15,16-tetranorlabd-7-en-(19,6*β*),(12,17)-diolide and *N. saprophytica* yielded four compounds, pitholide D, a cyclic derivative of pitholide, uridine and adenine. Nine compounds showed good antifungal activity against *C. clodosporioides* in TLC bio-autographic method. 1-(2,6-dihydroxyphenyl) butan-1-one and 5-hydroxy-2-methylchromane-4-one from *D. eschscholtzii*, 5-methylmellein, 8-methoxy-3,5-dimethylisochroman-1-one, 6,8-Dimethoxy-3-methylisocoumarin, reticulol and orcinol from *B. capnodes*, (*R*)-7-hydroxymellein, (*R*)-5-hydroxy mellein, 13,14,15,16-tetranorlabd-7-en-(19,6*β*), (12,17)-diolide, (3*R*,4*R*)-4,7-dihydroxy mellein from *N. parvum* and pitholide D, a cyclic derivative of pitholide, uridine and adenine from *N. saprophytica* have not been previously reported from the respective fungi. This study suggests that fungi *D. eschscholtzii*, *B. capnodes* and *N. parvum* are very good sources to produce small molecules with oxygen which are useful in synthetic organic chemistry as starting materials.

Conclusion

Four endophytic fungi isolated form the fruits of *P. acidus* (*D. eschscholtzii* and *B. capnodes*) and *E. serratus* (*N. parvum* and *N. saprophytica*) were identified by molecular levels. Secondary metabolites and compounds were isolated using several chromatographic separations. The compounds isolated from broth cultures of four different fungi were related to several chemical groups such as isocoumarins, melleine and naphthalene derivatives. *D. eschscholtzii*, *B. capnodes* and *N. parvum* are very good sources of heterocyclic oxygen containing small compounds which can be used in synthetic chemistry.

Selected References

Jayasinghe, L., Amarasinghe, N. R., Arundathie, B. G. S., Rupasinghe, G. K., Jayatilake, N. H. A. N., Fujimoto, Y. (2012). Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*, *Natural Product Research*, 26, 717–721.

Strobel, G., Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural product, *Microbiology and Molecular Biology Review*, 67, 491–502.

Yan, J., Xie, Y., Zhang, W., Wang, Y., Liu, J., Hyde, K. D., Seem, R. C., Zhang, G.-Z., Wang, Z.-Y., Yao, S.-W., Bai, X.-J., Dissanayake, A. J., Peng, Y.-L., Li, X.-H. (2013). Species of *Botryosphaeriaceae* involved in grapevine dieback in China, *Fungal Diversity*, 61, 221–236.

Zhang, Y. L., Ge, H. M., Zhao, W., Dong, H., Xu, Q., Li, S. H., Li, J., Zhang, J., Song, Y. C., Tan, R. X. (2008). Unprecedented immunosuppressive polyketides from *Daldinia eschscholzii*, a mantis-associated fungus, *Angewandte Chemie*, 120, 5823–5826.

Publications

Dissanayake D., Kumar N.S., Adikaram N.K.B., Jayasinghe L., Araya H. and Fujimoto Y. (2020). Production of small molecules by an endophytic fungus, *Neofusicoccum parvum* from the fruits of *Elaeocarpus serratus*. *Asian Journal of Traditional Medicines*, *15*(2).

Dissanayake D., Kumar N.S., Jayasinghe L., Araya H. and Fujimoto Y. (2019). Role of endophytic fungi in combating metabolic disorders. 9th ANRAP International Seminar; From Plant to Patients: Identification of new drugs for metabolic disorders, Pakistan.

Dissanayake D., Jayasinghe L., Kumar N.S., Fujimoto Y. and Araya H. (2018). Endophytic fungus, *Neofusicoccum parvum* from *Elaeocarpus serratus* – A source of small bioactive molecules. Asian Symposium on Medicinal Plants, Spices and Other Natural Products (ASOMPSxvi), Sri Lanka.

Dissanayake D., Munasinghe M.V.K., Qader M.M., Rathnayake G.R.N., Sritharan T., Thanabalasingam D., Padmathilake K.G.E., Bandara H.M.S.K.H., Adikaram N.K.B., Jayasinghe L., Kumar N.S., Fujimoto Y. and Araya H. (2017). Fungi from edible fruits as a source of bioactive compounds. 1st Sri Lankan ANRAP Regional Seminar (ANRAPSL1), National Institute of Fundamental Studies, Kandy, Sri Lanka, P. 66.

Dissanayake D., Jayasinghe L., Kumar N.S. and Fujimoto Y. (2016). Phenolic metabolites from an endophytic fungus *Daldinia eschscholtzii* from the fruits of *Phyllanthus Acidus*. Postgraduate Institute of Science Research Congress, University of Peradeniya, Peradeniya, Sri Lanka, P. 105.

Dissanayake D., Jayasinghe L., Kumar N.S. and Fujimoto Y. (2016). A series of isocoumarins from an endophytic fungus *Biscogniauxia capnodes* from *phyllanthus acidus*. 4–5 Nov 2016, University of Peradeniya International Research Sessions (iPURSE), University of Peradeniya, Peradeniya, Sri Lanka, P. 316.

Dissanayake D., Jayasinghe L., Kumar N.S. and Fujimoto Y. (2016). Endophytic fungi; A potential source for oxygen containing heterocyclic compounds, 19-23 Sep 2016, National Workshop on Separation Techniques in Natural Products Research, National Institute of Fundamental Studies, Kandy, Sri Lanka.

Thesis Reference

Dissanayake D.M.D.M. (2019). Chemistry and Bioactivity of Secondary Metabolites Produced by Endophytic Fungi in the Fruits of *Phyllanthus acidus* and *Elaeocarpus serratus*. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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DEVELOPMENT OF ADSORBENTS FOR TEXTILE DYE AND HEAVY METAL CONTAMINATED WASTEWATER TREATMENT

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Small and medium-size enterprises (SMEs) in developing countries use textile dyes in their textile industries and heavy metals such as chromium in leather tanning. Due to economic constraints, SME owners do not invest in expensive wastewater treatment systems. Therefore, it is required to identify a cost-effective physio-chemical method to address this issue. The use of biological materials as an adsorbent is being practiced for decades, hence we studied the behavior of selected invasive plants and fern species to adsorb heavy metals and textile dyes from synthetic wastewater. Going one step beyond, we used the biopolymers available in the market and synthesized polymer-layer silicate composite adsorbents by combing the polymer substrate with naturally available clay, kaolinite which is more durable than the plant materials. The metal organic frameworks were synthesized using green synthesis methods to reduce energy used and the time used in the synthesis process.

The investigation for an adsorbent was based on three main objectives:

(I) To identify a new low-cost adsorbent material for heavy metal and textile dye contaminated wastewater treatment

(II) To use polymer layer silicates for heavy metal and textile dye removal

(III) To use Metal Organic Frameworks (MOFs) for heavy metal and textile dye removal

Adsorbtion experiments were performed using two plant species, two polymer-layer silicate composites and a metal organic framework. Their preparation is given as follows.

Methodology

Preparation of biosorbents

Fresh *Mimosa pigra* L. seed pods and *Asplenium nidus* L. leaves were collected from Mahaweli riverbanks in the Digana area and Gampola area, respectively. Initially, plants were authenticated at the National Herbarium of the Royal Botanical Gardens, Peradeniya. Thereafter, plants were thoroughly cleaned with running tap water, followed by deionized water. The biomass was

air-dried for two days and oven-dried at 70 °C for two days. The dried biomass was ground using a commercial grinder to obtain particle fractions with a diameter between 250 µm and 350 µm through sieving.

Preparation of composite adsorbents

Analytical grade sodium alginate (1.0 g) (SRL, India) and 1.0 g of chitosan (Sigma Aldrich Germany) were dissolved in 150 mL of distilled water at 80 °C and 300 mL of 1% acetic acid, stirred for 2 h and 48 h, respectively. Thereafter, 20.0 g of kaolin was added to the sodium alginate and chitosan solutions separately and stirred for 6 h and 48 h, respectively. Then sodium alginate mixture was added to 1% $CaCl_2$ solution using a syringe. Similarly, a chitosan mixture was added to a 0.5 mol L^{-1} NaOH solution to form beads. Beads formed were allowed to rest in the solution for another 48 h. The beads were separated from the cross-linking solution and washed with distilled water until the water is neutral. Beads were air-dried for 48 h and oven-dried for 48 h. Dried beads were ground to obtain a particle size 250-350 µm.

Synthesis of MIL 53(Fe)

Iron(III) chloride (10 mmol) and benzene-1,4-dicarboxylic acid (10 mmol) were dissolved separately in 50 mL of N,N-dimethylformamide (DMF). The solutions were stirred for 1 h, and the two solutions were mixed and stirred for 1 h. The mixture was transferred to Teflon vessels and the vessels were loaded into a microwave digester (Milestone Start D). The final temperature of the mixture was brought up to 150 °C within 5 min. The mixture was kept at 150 °C for 25 min. After the mixture reached room temperature (30 °C) the Teflon vessels were opened and the MIL 53(Fe) was collected. MIL 53(Fe) was washed three times with DMF and finally with ethanol and oven-dried at 70 °C for 24 h. Synthesized MIL 53(Fe) was stored.

Adsorption Experiments

A dosage of 2.00 g L^{-1} of adsorbent material was introduced into 100.0 mL of 5.0 mg L^{-1} metal or textile dye solution at an initial pH of 5.0 and ambient temperature 27 °C. Suspensions were shaken on an orbital shaker at a rate of 100 rpm. Suspensions were removed from the shaker at predetermined time intervals and suspensions were filtered using Whatman No.1 filter paper. After discarding a few mL of filtrate initially, the rest was collected and analyzed using AAS or UV-Vis for residual metal or dye concentration. The same procedure was repeated for other experimental parameters changing the one parameter at a time.

Temperature programmed desorption of aniline and benzene from functionalized silica surfaces

A home-built UHV chamber was used for the experiments, equipped with a residual gas analyzer, dosing system with heatable nozzle, ion imaging system, and laser detection system. Laser ionization was performed using a nanosecond pulsed dye laser (Sirah, Precision Scan), which was pumped by the third harmonic of an Nd:YAG laser (Spectra-Physics, Quanta Ray 250-10). In some experiments, aniline was ionized using the fundamental frequency (790 nm) of a femtosecond laser system (Clark-MXR).

Results and Discussion

Biosorption of metals

Biosorption of the metal ions onto the *Mimosa pigra* and *Asplenium nidus* with the time of contact remained constant after reaching equilibrium. The time taken for maximum biosorption depended on the metal species. The maximum biosorption of Pb(II) was 63% after 90 min and for Ni(II) 45% after 30 min for *M. pigra* and. Pb(II) was 95% after 75 min and for Ni(II) 58% after 30 min for *A. nidus* biosorbents (figure 1).

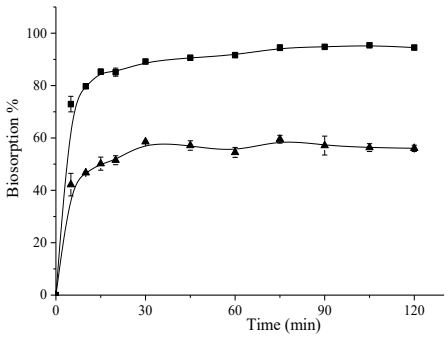

Figure 1. Effect of contact time on Pb(II) and Ni(II) biosorption by 0.20 g of *A. nidus* biosorbent at pH 5.0 at 27 °C (initial metal concentration = 5.0 mg L^{-1}, shaking speed 100 rpm, Pb(II) = ▬■▬, Ni(II) = ▬▲▬ , n = 3)

The optimization of the biosorption process showed that the *A. nidus* biosorbent is a potential candidate for Pb(II) and Ni(II) adsorption (table 1).

The desorption of adsorbed heavy metals is an important aspect of biosorption. In this study, we

used cost-effective desorbents to recover heavy metals from the biosorbent. It was found that Na_2CO_3 and CH_3COONa can be used to recover Pb(II) and Ni(II) respectively (Figure 2).

Table 1. Biosorption parameters of Pb(II) and Ni(II) biosorption by *M. pigra* and *A. nidus* biosorbent

Adsorbent	Parameter	Pb(II)	Ni(II)
M. pigra	Adsorption %	63	45
	pH	4-6	
	Isotherm	sips	Langmuir-Freundlich
	Kinetics	Pseudo 2nd	Pseudo 1st
A. nidus	Adsorption %	95	58
	pH	4-6	
	Isotherm	Langmuir-Freundlich	
	Kinetics	Pseudo 2nd	

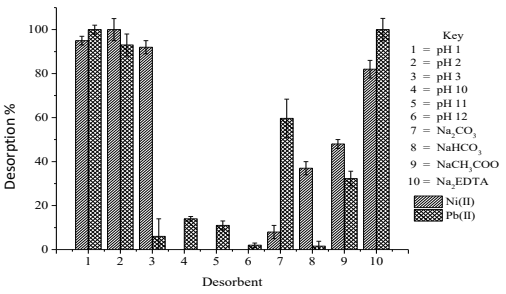

Figure 2. Percentage desorption of Ni(II) and Pb(II) by different desorbents from 0.10 g of metal loaded *A. nidus* biosorbent (temperature 27 ºC, shaking speed = 100 rpm, Ni(II) ▨, Pb(II) ▩, n = 3)

Polymer layer silicate composite adsorbents

Polymer layer silicate materials prepared from kaolin-alginate and kaolin-chitosan were used to remove fuchsin, methylene blue, and Cr(III). As shown in the table 2, kaolin-alginate composites can remove over 90% of fuchsine, methylene blue, and Cr(III). Similaraly, kaolin-chitosan composite also showed 90% removal. However, the kaolin-alginate composite adsorbent is more suitable since it can be used in a wide range of pH than the kaolin-chitosan composite.

Table 2. Fuchsine, methylene blue, and Cr(III) adsorption onto the kaolin-alginate adsorbent

	Fuchsine	Methylene blue	Cr(III)
Adsorption %	93	97	97
Equilibrium time (min)	240	90	120
Optimum pH	3-9	3-9	4-7

Metal Organic Frameworks for dye remediation

The metal organic frameworks (MOFs) are crystalline compounds that contain a metal cluster and an organic linker. High surface area and the porosity of metal organic frameworks enable the high adsorption of pollutants from aqueous systems.

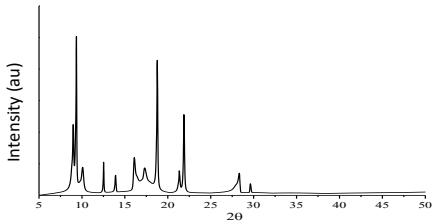

Figure 3. XRD spectrum of MIL 53(Fe) main peaks is observed at 9.36, 12.52, 16.12, 17, 38, 18.78, 21.88, 28.28

MIL 53(Fe) is a Fe based metal organic framework used in adsorption of methylene blue. The XRD pattern of the MIL 53(Fe) used in the experiment is shown in the figure 3. It adsorbed, 85% of methylene blue after 150 min within the pH range of pH 4-8.

Temperature programmed desorption of aniline and benzene from functionalized silica surfaces

Laser-assisted temperature probed desorption experiments suggest that main functional groups of dyes, aniline, and benzene are having different interactions with the functionalized silica surfaces.

Table 3. Parameters for aniline desorption from functionalized silica surfaces

	AFS	CFS
Desorption Temperature	149 K	170-174 K
Desorption Energy	41.00 kJ mol^{-1} (0.37 eV)	47.46 kJ mol^{-1} (0.41 eV)

From the laser-assisted temperature programmed desorption studies, ─NH_2 surface form π-lone pair interactions with benzene and H-bond with anilines whereas, ─COOH surface forms π-lone pair interactions with benzene and acid-base interactions, H-bonding with anilines. Adsorption energy calculations (results are shown in the table 3) suggest that these interactions are responsible for methylene blue and fuchsine adsorption onto polymer layer silicates.

Conclusion

In conclusion, the polymer layer silicates are more environmentally friendly and effective in removal of both textile dyes and heavy metals than the plant materials per se. The MIL 53(Fe) can be used to remove Cr(VI), which could not be removed using polymer layer silicates. A composite of MIL 53(Fe), kaolin and alginate or chitosan can be used as a universal adsorbent to remove both cationic and anionic pollutants. The laser ionization assisted temperature programmed desorption can be used to determine the interactions between the adsorbate and the adsorbent.

Source of Funding

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Erasmus Mundus SmartLink Program

Selected References

Sarkar, B. (2002). *Lead*, in *Heavy Metals In The Environment*, E.F. Madden, et al., Editors., CRC Press.

Rowsell, J. L. C., and Yaghi, O. M. (2004). Metal–organic frameworks: a new class of porous materials. *Microporous and Mesoporous Materials*, 73, 3-14.

Ho, Y. S. and McKay, G. (1998). A comparison of chemisorption kinetic models applied to pollutant removal on various sorbents. *Process Safety and Environmental Protection*, 76, 332-340.

Huang, L.-z., Zeng, G.-m., Huang, D.-l., Li, L.-f., Du, C.-y., and Zhang, L. (2010). Biosorption of cadmium (II) from aqueous solution onto *Hydrilla verticillata*. *Environmental Earth Sciences,* 60, 1683-1691.

Schroeder, S. L. and Gottfried, M. (2002) Temperature-Programmed Desorption (TPD) Thermal Desorption Spectroscopy (TDS). *Userpage. Chemie. Fu-Berlin. De*, 1-22.

Publications

Dissanayake, D. M. R. E. A, Wijesinghe, W.M.K.E.H, Iqbal, S.S, Priyantha, H.M.D.N and Iqbal, M. C.M. (2016). Fuchsine biosorption using *Asplenium nidus* L. biosorbent- A mechanism using kinetic and isotherm data. *RSC Advances*. 6: 101, 98682-98692.

Dissanayake, D. M. R. E. A, Wijesinghe, W.M.K.E.H, Iqbal, S.S, Priyantha, H.M.D.N and Iqbal, M. C.M. (2016). Isotherm and kinetic study on Ni(II) and Pb(II) biosorption by the fern *Asplenium nidus* L. *Ecological Engineering.* 88:237-241.

Dissanayake, D.M.R.E.A., Iqbal, S.S, Priyantha, H.M.D.N and Iqbal, M. C.M. (2019). Green synthesis of MIL 53(Fe) and its use as an effective adsorbent of cationic dyes, Conference Abstracts, Green & Sustainable Chemistry Conference, Intercontinental Hotel Berlin, Germany.

Dissanayake, D.M.R.E.A., Iqbal, S.S., Priyantha, N. and Iqbal, M. C.M. (2018). The adsorption of Cr(III) onto Kaolin: Alginate composite adsorbent. Symposium Abstract. *Open University International Research Sessions*. Open University of Sri Lanka, Nawala.

Thesis Reference

Dissanayake, D. M. R. E. A**,** **(**2019). Development of Adsorbents for Heavy Metal and Dye Contaminated Wastewater Treatment. Ph.D. Thesis, University of Peradeniya.

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CHEMISTRY AND BIOACTIVITY OF FIVE POPULAR EDIBLE FRUITS IN SRI LANKA

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Fruits are a promising source for the identification of environmental friendly bioactive compounds. Being a tropical country, Sri Lanka is rich in many edible fruits throughout the year. However, most of the fruits are underutilized. Five fruits, namely, *Citrullus lanatus* (Thunb.), *Limonia acidissima* L., *Nephelium lappaceum* L., *Passiflora edulis* Sims. and *Phyllanthus emblica* L. were selected for the study.

The study was carried out with following objectives:

(I) To identify fruits with α-amylase enzyme inhibitors, allelopathic, antioxidant, antifungal and cytotoxic properties

(II) To sub-fractionate bioactive extracts in selected fruits

(III) To identify compounds present in selected fruits

Methodology

Edible parts of well ripened fruits were blended and filtered. The filter cake was sequentially extracted into *n*-hexane (RH), ethyl acetate (RE) and methanol (RM). The aqueous filtrate was successively partitioned with *n*-hexane (JH) and ethyl acetate (JE) and the resulting aqueous fraction (JW) was freeze dried. Part of the concentrated methanol extract of the residue was partitioned with *n*-butanol (RMB) and water (RMW) and the water fraction was freeze dried. The concentrated extracts were subjected to bioactivity studies. Bioactive extracts were sub-fractionated to yield four high performance liquid chromatography (HPLC) and two preparative thin layer chromatography (PTLC) fractions from ethyl acetate extract of *L. acidissima* and two PTLC fractions from ethyl acetate extract of *P. edulis* which were further subjected to bioactivity studies. 70% methanol/H_2O extracts of the fruits were subjected to LC-MS studies.

Bioassays

The extracts were subjected to bioactivity studies including allelopathic activity against lettuce (*Lactuca sativa*) seeds (Salam *et al.*, 2010), α-amylase enzyme (porcine pancreas) inhibitory assay (Nickavar *et al.*, 2008), anticandidal activity against *Candida albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) and *C. tropicalis* (ATCC 13803) (Andrews, 2001), antifungal activity against *Cladosporium cladosporioides* (Morandim *et al.*, 2010), antioxidant activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Aliyu *et al.*, 2009) and brine shrimp lethality assay against *Artemia salina* (Krishnaraju *et al.*, 2005).

Results and Discussion

Table 1 shows the results of bioassays conducted on fruit extracts. Among all the extracts of *C. lanatus* (Thunb.) fruits, the lowest IC_{50} values for seed germination inhibition was reported by JH (395±6 mg/L) and for DPPH radical scavenging was given by JE extract (1012±0.1 mg/L). Inhibition of *L. sativa* seed germination and inhibition of *C. cladosporioides* by *C. lanatus* fruit extracts were reported for the first time.

IC_{50} value for inhibition of seed germination by *L. acidissima* L. JE extract was 766±4 mg/L. the same extract showed the highest α-amylase enzyme inhibitory effect with IC_{50} value of 975±2 mg/L and the lowest LD_{50} value (28±0.3 mg/L) against brine shrimp lethality assay among all the *L. acidissima* L. extracts. RMW exhibited the highest DPPH radical scavenging activity with IC_{50} value of 401±1 mg/L among all extracts of *L. acidissima* L. JE extract of *L. acidissima* L. was active against *C. parapsilosis*. This is the first report of *L. acidissima* fruit extracts inhibiting the germination of *L. sativa* seeds and activity against *C. parapsilosis.* Two sub-fractions from ethyl acetate extract of *L. acidissima* exhibited antioxidant activity.

RE and JE extracts of *N. lappaceum* L. had IC_{50} values of 1051±0.6 mg/L and 955±1 mg/L against DPPH radical scavenging activity respectively. Antifungal effects against *C. cladosporioides* and lethal effects against *A. salina* of *N. lappaceum* fruit extracts were reported for the first time. The lowest IC_{50} value (635±2 mg/L) for inhibition of root elongation was given by RE extract among all the extracts of *P. edulis* Sims. This is the first report of *P. edulis* fruit extracts showing inhibition of *L. sativa* root elongation, inhibition of α-amylase enzyme and lethal effects on *A. salina.*

The lowest IC_{50} value (929±2 mg/L) for root elongation inhibition was reported by RE extract among all the extracts of *P. emblica* L. Inhibition of *C. parapsilosis* by *P. emblica* fruit extracts was reported for the first time.

Table 1. Results of bioassays

	Allelopathic	α-Amylase inhibitory	Anticandidal	Antifungal	Antioxidant	Brine shrimp lethality
C. lanatus	JH, JE (seed germination)	-	-	RH, RE, JH, JE	JE	RE, JH, JE
L. acidissima	JE (seed germination)	RE, RMB, JE	JE	-	RE, RM, RMB, RMW, JE	RE, RM, RMB, RMW, JE, JW
N. lappaceum	-	JE	-	RH, RE, JH	RE, JE	RH, RE, RMB, JE
P. edulis	RE, JW (root elongation)	RE,JE	-	-	RH, RE, RM, RMB, RMW, JH, JE	RE, RM, RMB, RMW, JH, JE, JW
P. emblica	RE, JE, JW (root elongation)	RE, RM, RMB, JE, JW	RMB	-	RH, RE, RM, RMB, RMW, JH, JE, JW	RE, RM, RMB, RMW, JE, JW

LC-MS studies showed the presence of malic acid, quinic acid, protocatechuic acid pentoside (first report) and monohydroxybenzoyl hexoside (first report) in *C. lanatus,* monohydroxybenzoic acid, dihydroxybenzoic acid (first report) and monohydroxybenzoyl hexoside (first report) in *L. acidissima* and methyl gallate and monogalloylhexose in *P. emblica.*

Conclusion

All the fruits contained bioactive extracts. LC-MS studies indicated the presence of malic acid, quinic acid, protocatechuic acid pentoside and monohydroxybenzoyl hexoside in *C. lanatus,* monohydroxybenzoic acid, dihydroxybenzoic acid and monohydroxybenzoyl hexoside in *L. acidissima* and methyl gallate and monogalloylhexose in *P. emblica.*

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Selected References

Aliyu, A.B., Musa, A.M., Ibrahim, M.A., Ibrahim, H. and Oyewale, A.O. (2009). Preliminary phytochemical screening and antioxidant activity of leave extract of *Albizia chevalieri* Harms (Leguminoseae-Mimosoideae). *Bayero Journal of Pure and Applied Sciences* 2, 149–153.

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 48, 5–16.

Krishnaraju, A. V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H.-S. and Subbaraju, G. V. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering* 3, 125–134.

Morandim, A.D.A., Pin, A.R., Pietro, N.A.S., Alecio, A.C., Kato, M.J., Young, C.M., Oliveira, J.E. De. and Furlan, M. (2010). Composition and screening of antifungal activity against *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* of essential oils of leaves and fruits of *Piper* species. *African Journal of Biotechnology* 9, 6135–6139.

Nickavar, B., Abolhasani, L. and Izadpanah, H. (2008). α-Amylase inhibitory activities of six *Salvia* species. *Iranian Journal of Pharmaceutical Research* 7, 297–303.

Salam, A. and Kato-noguchi, H. (2010). Evaluation of allelopathic potential of Neem (*Azadirachta indica*. A. Juss) against seed germination and seedling growth of different test plant species. *International Journal of Sustainable Agriculture* 2, 20–25.

Publications

Niyangoda, D., Kumar, N.S. and Jayasinghe, L. (2019). Edible fruits as a source of drug candidates, MMDR-7, Karachi, Pakistan, 131.

Niyangoda, D., Kumar, N.S. and Jayasinghe, L.(2018). Bioactivity studies of *Citrullus lanatus* fruit pulp, ASOMPSS XVI 2018, Colombo, Sri Lanka, 177.

Niyangoda, D., Kumar, N.S. and Jayasinghe, L. (2018). α-Amylase inhibitory effects of common edible fruit extracts, ANRAPSL-1 – Herbal approaches in combating diabetes and common tropical diseases, NIFS, Kandy, Sri Lanka, 52.

Niyangoda, D., Kumar, N.S. and Jayasinghe, L. (2016). Allelopathic activity of some edible fruit extracts against *Lactuca sativa*, Proceedings of the *i*PURSE, Sri Lanka, 20, 343.

Thesis Reference

Niyangoda, H.R.W.M.D.P.K. (2019). Chemistry and Bioactivity of Five Popular Edible Fruits in Sri Lanka. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

Institute where research was carried out

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MANUFACTURING OF MULTI-FUNCTIONAL COTTON AND POLYESTER FABRICS USING NANO-TECHNOLOGICAL ARCHITECTURES

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There has been lot of work reported on developing smart textile materials. However, reports on reliable methods which can be integrated to industrial scale manufacture and textiles with more than one self-cleaning capabilities are lacking. Therefore, development of different types of self-cleaning textile materials integrating more than one self-cleaning process was attempted in this study. The study was carried out with following objectives:

~~(I)~~ To investigate and characterize the attachment of TiO_2 and ZnO nano-materials on large cotton pieces

(II) To investigate the anti-bacterial characteristics of modified textile materials

(III) To self-assemble fluorinated fatty acid molecules on nano-architectures and to investigate the super-hydrophobic properties

(IV) To design and develop intelligent stain-resistant cotton/polyester fabrics

(V) To investigate the integration of modified textiles for industrial applications

Methodology

Two types of textile materials; cotton and polyester were functionalized with TiO_2 and ZnO nano-structures together with other organic molecules to integrate the self-cleanings properties.TTIP and chloroacetic acid were used as the starting precursors and ethanol as a solvent to prepare TiO_2 nano-particles. $Zn(NO_3)_2$ mixed with Hexamethyltetra ammine in ethanol to prepare ZnO nano-structures. Synthesized nano-materials were attached to the fabric with PVA using a dipping method. Stearic acid in ethanolic solution was used as the surface modifier to obtain super-hydrophobic properties. Developed materials were characterized using X-ray Diffraction (XRD), Scanning Electron Microscope (SEM) and UV-Visible spectroscopy. Chemical degradation of stains was characterized with UV-Visible reflectance data and the super-hydrophobicity of the modified fabrics was determined with respect to their water contact angle measurements. Conventional spread plate techniques were carried out to identify the

antibacterial properties. The industrial scale and commercial scale manufacturing (over 100 m of fabric) was carried out in the Textured Jerseys Lanka Pvt. Ltd, Awissawella.

Results and Discussion

XRD pattern of the powdered TiO_2 nano-particles prepared for making the seed layer on cotton is illustrated in figure 1. The major peak at 2θ value of 25.73º indicates the presence of TiO_2 in the form of rutile. The estimated average crystallite size from Debye-Scherrer formula is 31.02 nm.

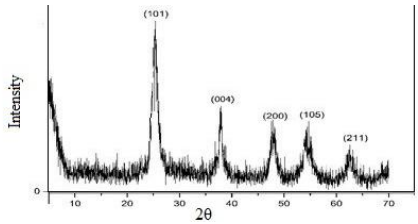

Figure 1. XRD pattern of TiO_2 nano-particles prepared for the seed layer

XRD pattern of cotton/TiO_2 seed particles/TiO_2/ ZnO composite is given in figure 2 which confirms the presence of both TiO_2 and ZnO since peaks at 25.51° and 36.13° represent rutile form of TiO_2 and wurtzite form of ZnO

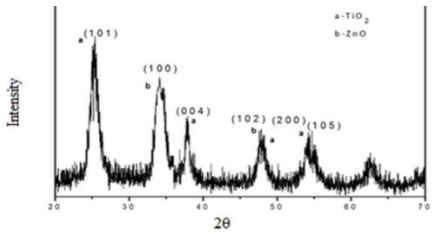

Figure 2. XRD pattern of cotton/TiO_2 seed particles/TiO_2/ZnO composite

As illustrated in the figure 3 the modified fabric showed a strong absorption of visible light in the wavelength range of 400-500 nm, indicating the visible light activity due to the modification. This can be attributed to the formation of a dipole layer subsequent to the reaction between the carboxyl groups from stearic acid and the hydroxyl groups of TiO_2 causing the reduction of the band gap of TiO_2. This causes a much easier photo-catalytic degradation of the organic stains and microbial cells inducing the self-cleaning and anti-microbial activities. ZnO absorb the photons and cause the excitation of electrons from the valence band to the conduction band similar to TiO_2 following the generation of electron-hole pairs leading to the formation of hydroxyl radical species that are capable of reacting with the organic stains and organic components of the microbial cell membranes following their degradation.

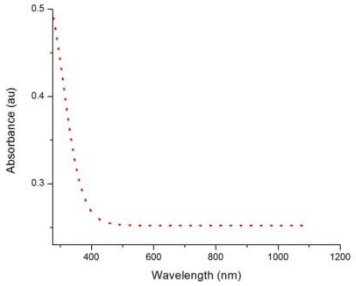

Figure 3. UV-Visible spectrum for undyed cotton fabric, modified with TiO_2/ ZnO nano-composite

The photo-catalytic degradation of the organic stains was evaluated over the modified fabrics and non-modified fabrics using the same method described previously. During photo-catalytic degradation experiments, the intensities of the organic stains on modified fabrics were found to decrease upon solar irradiation time, confirming that photo-degradation of stains takes place (figure 4). Under UV light irradiation, non-modified fabrics had no effect towards any organic stain even after 20 h (figure 5).

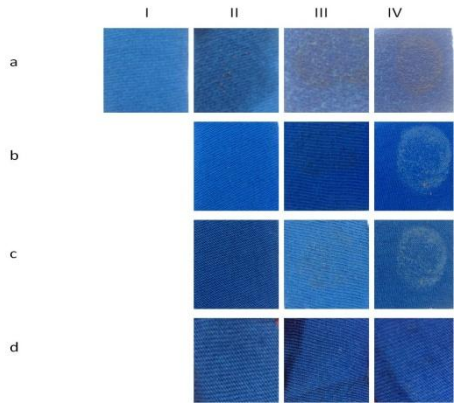

Figure 4. Images of dyed, modified, cotton fabric pieces stained with (a, I) none, (a, II) tea, (a, III) coffee, (a, IV) ketchup, before exposing to sun, (b, II) tea, (b, III) coffee, (b, IV) ketchup, after exposing 5 h to sun, (c, II) tea, (c, III) coffee, (c, IV) ketchup, after exposing 10 hours to sun, (d, II) tea, (d, III) coffee, (d, IV) ketchup, after exposing 20 h to sun

The water contact angles were measured with respect to the number of washing cycles. As shown in the table 1, both laboratory and industrially modified fabrics achieve much higher values for the water contact angles, indicating their super-hydrophobic property even up to 45 washing cycles.

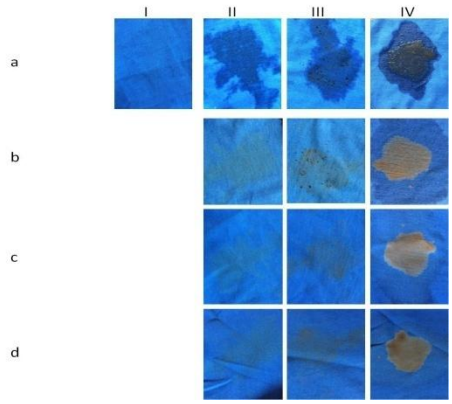

Figure 5. Images of dyed, none-modified, cotton fabric pieces stained with (a, I) none, (a, II) tea, (a, III) coffee, (a, IV) ketchup, before exposing to sun, (b, II) tea, (b, III) coffee, (b, IV) ketchup, after exposing 5 h to sun, (c, II) tea, (c, III) coffee, (c, IV) ketchup, after exposing 10 hours to sun, (d, II) tea, (d, III) coffee, (d, IV) ketchup, after exposing 20 h to sun

Table 1: Water contact angles of modified fabrics with number of washing cycles

Laboratory modified cotton fabric		Laboratory modified polyester fabric		Industrially modified cotton fabric		Industrially modified polyester fabric	
Number of washing cycles	Water contact angle	Number of washing cycles	Water contact angle	Number of washing cycles	Water contact angle	Number of washing cycles	Water contact angle
0	156°	0	153°	0	153°	0	151°
5	154°	5	152°	5	152°	5	151°
10	153°	10	151°	10	151°	10	151°
20	152°	20	151°	20	151°	20	150°
30	152°	30	150°	30	151°	30	148°
40	151°	40	150°	40	150°	40	145°
45	151°	45	150°	45	148°	45	142°

This confirms even after 45 washing cycles, the nano-materials remain on the fabric due to the attachment of TiO_2 nano-particles by PVA. As such, garments made of these modified fabrics can be worn several times without washing every time after wearing. The SEM images in figures 6 (a) and (b), related to the laboratory modified and industrially modified fabrics reveal that rod like nano-structures are grown on the fabrics, and these hierarchical structures provide desiring nano roughness to the fabric surface to have the super-hydrophobic property. The method is industrially applicable and time efficient, and 100 m of commercial scale production was successfully delivered in the industry for both cotton and polyester fabrics.

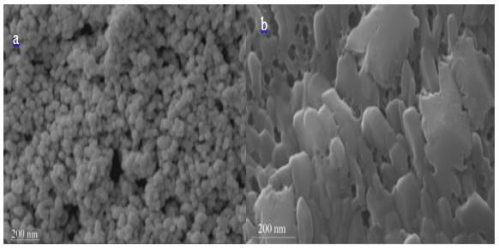

Figure 6. SEM images of (a) TiO_2 nano-particles in seed layer, (b) TiO_2 nano-protrude structures and ZnO nano-structures on fabric

In this method, growth inhibition of bacteria can be due to chemical as well as physical reasons. If the size of TiO_2 nano-rods is very small like in this case, the tip of the nano-rods is around 50 nm, it can penetrate the cell membrane leading to its disintegration and malfunctioning of the permeability barrier, which cause the death of bacteria. The second important reason for bacterial growth inhibition by TiO_2 and ZnO is the photocatalytic production of Reactive Oxygen Species (ROS). The ROS include $\cdot OH$, H_2O_2 and O_2^{2-}.

Bacteria carry negative charge on the surface and therefore, the penetration of O_2^{2-} seems impossible but the hydroxyl radical and hydrogen peroxide can penetrate into the cell membrane which leads to the death of bacteria. Due to this composite system the fabric can obtain the desiring property even in dark conditions.

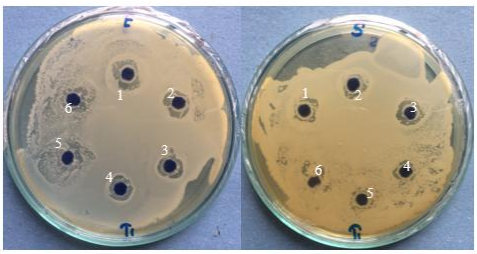

Figure 7. (a) A week-aged modified cotton fabric piece, after (1) 0 washing cycles (2) 5 washing cycles, (3) 10 washing cycles (4) 20 washing cycles (5) 30 washing cycles (6) 40 washing cycles, showing the respective inhibition zones against *Escherichia coli*. (b) A week-aged

laboratory modified cotton fabric piece, after (1) 0 washing cycles (2) 5 washing cycles, (3) 10 washing cycles (4) 20 washing cycles (5) 30 washing cycles (6) 40 washing cycles, showing the respective inhibition zones against *Staphylococcus aureus*

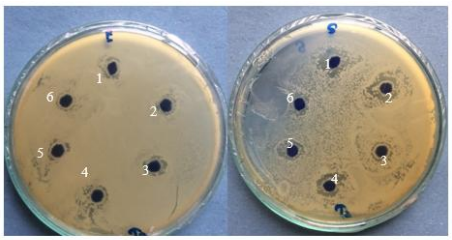

Figure 8. (a) A week-aged modified polyester fabric piece, after (1) 0 washing cycles (2) 5 washing cycles, (3) 10 washing cycles (4) 20 washing cycles (5) 30 washing cycles (6) 40 washing cycles, showing the respective inhibition zones against *Escherichia coli*. (b) A week-aged laboratory modified polyester fabric piece, after (1) 0 washing cycles (2) 5 washing cycles, (3) 10 washing cycles (4) 20 washing cycles (5) 30 washing cycles (6) 40 washing cycles, showing the respective inhibition zones against *Staphylococcus aureus*

As shown in the above figure 7 and 8, both types of fabrics (cotton and polyester) are capable of inhibiting the growth of bacteria in their vicinities, after 24 hours of incubation. And the property lasts for more than 40 washing cycles and they can inhibit both Gram positive and Gram negative bacterial strains. The whole system can perform the desiring property in both dark and light conditions; still anti-microbial activity analyzing was carried out in dark conditions to confirm the ability of modified fabrics to destroy the microbial cells mechanically without any support of UV or visible light.

Conclusion

The nano-materials which are highly mono-crystalline were successfully grown on micro fibers of cotton and polyester fabrics. The nano-materials strongly bind with the fabric with anchoring agents such as PVA, and that increases the durability of the fabric. The nano-materials treated fabrics have the ability to degrade the colour stains with which it comes in contact. The functionalized fabrics have biological self-cleaning. The treated fabrics not only prevent the growth of bacteria but also kill the ones which attach on it. The method was successfully carried out in the industry, manufacturing multifunctional smart fabrics in commercial scale (100 m).

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Selected References

Guo, Z. and Liu, W. (2007). Biomimic from the superhydrophobic plant leaves in nature: binary structure and unitary structure. *Plant Science*, 172(6), 1103-1112.

Morkoç, H. and Özgür, Ü. (2009). *General properties of ZnO, Zinc oxide: fundamentals, materials and device technology*, Wiley -VCH Verlag Gmb H & Co. KGaA.

Kiwi, J. and Pulgarin, C. (2010). Innovative self-cleaning and bactericide textiles. *Catalysis Today*, 151(1-2), 2-7.

Selected Publications

Dunuweera, S.P., Rajapakse, R.M.S.I., Rajapakshe, R.B.S.D., Wijekoon, S.H.D.P., Thilakarathna, N., Sasanka, M.G.G. and Rajapakse, R.M. (2019). Review on targeted drug delivery carriers used in nanobiomedical applications. *Current Nanoscience*, 15(4), 382-397.

Rajapakshe, R.B.S.D., Thennakoon, C.A., Rajapakse, R.M.G. and Rajapaksha, S. (2018). Multi-functional cotton fabrics with self-assembled TiO_2 nanoparticle seed/TiO2 nanorod/ZnO nanoparticle/stearic acid nanotechnological architectures, *Journal of Nanomaterials and Molecular Nanotechnology.*

Thesis Reference

Rajapakshe R.B.S.D. (2019). Scaling Up The Process of Developing Self-cleaning and Antibacterial Textile Materials. Ph.D. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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INVESTIGATION OF ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF SELECTED SRI LANKAN GROWN SPICES AS POTENTIAL THERAPEUTIC AGENTS FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is one of the age related progressive neurodegenerative diseases that affects memory and cognitive behavior. In Sri Lanka prevalence and incidence of AD is projected to be 148% and 45.1% respectively in 2020. Therefore, there is an urgent need for the disease management in Sri Lanka as well as in worldwide. There is no cure for AD, symptomatic treatment includes the use of anticholinesterases. Therefore, there is a need to discover new acetylcholinesterase inhibitors with less side effects, increased efficacy and bioavailability. Hence this study was aimed at investigating natural anticholinesterases and bioactive compounds in Sri Lankan spices: Seeds of *Brassica juncea* (mustard), *Coriandrum sativum* (coriander), *Trigonella foenum-graecum* (fenugreek), *Foeniculum vulgare* (fennel), and *Piper nigrum* (pepper); leaf stalks of *Cymbopogon citratus* (lemongrass); fruit pod *Elettaria cardamomum* (cardamom); flower bud of *Eugenia caryophyllus* (clove), dried fruit rind of *Garcinia cambogia* (garcinia); fruit aril of *Myristica fragrans* (mace); fruit and seeds of *Tamarindus indica* (tamarind); rhizome of *Zingiber officinale* (ginger).

Figure 1. A: Fruit of *M. fragrans*; **B**: Cross section of the fruit with fruit aril of *M. fragrans* (mace)

The study was carried out with following objectives:

(I) To investigate acetylcholinesterase (AChE) inhibitory activity of selected spices grown in Sri Lanka

(II) To determine the AChE inhibitory, antioxidant and antidiabetic activity and

antifungal activity of pure compounds isolated from mace

(III) To study the structure activity relationship of the most active isolate

Methodology

n-Hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) extracts of spices prepared from sequential extraction were screened for acetylcholinesterase (AChE) inhibitory activity using Ellman's method with slight modifications. Extract with the highest anticholinesterase activity was chromatographed and compounds isolated were screened for anticholinesterase, anti-amylase, anti glucosidase and antioxidant activity. Most anticholinesterase active compound was investigated for structure activity relationship.

Results and Discussion

M. fragrans, *G. cambogia, and T. indica* seed showed AChE inhibitory activity with IC_{50} values: *M. fragrans* IC_{50}: *n*-hexane- 29.03±0.11 ppm, DCM- 21.37± 0.07 ppm, EtOAc- 18.29±0.04 ppm, MeOH- 13.44±0.13 ppm (table 1 shows percentage AChE inhibition of *M. fragrans* extracts at the concentration of 100 µg/mL); *G. cambogia* IC_{50}: *n*-hexane- 42.74±0.10 ppm, DCM- 61.44± 0.08 ppm; *T. indica* seed IC_{50}: MeOH- 15.88±0.01 ppm (positive control donepezil hydrochloride IC_{50}; 0.03± 0.00 ppm). MeOH and EtOAc extracts of fruit aril of *M. fragrans* were combined owing to the similar pattern observed in TLC and purified using various chromatographic techniques to afford 6 compounds: malabaricone C (1), 3'-methyl-5'-pentyl-furylarylic acid (2), a fatty acid (3), licarin A (4), elemicin (5) and 5-methoxylicarin B (6) which were screened for antioxidant, AChE inhibitory, *α*-amylase inhibitory, *α*-glucosidase inhibitory (table 2) and antifungal activities. Malabaricone C showed highest AChE inhibitory activity of IC_{50} 2.06±0.04 ppm and antioxidant activity of 6.56±0.02 ppm. 3'-Methyl-5'-pentyl-furylarylic acid and fatty acid showed *α*-glucosidase inhibitory activity of IC_{50} 51.02±0.01 ppm and 46.74±0.01 ppm respectively. Antifungal activity against *Cladosporium cladosporioides* was observed in malabaricone C, elemicin and 3'-methyl-5'-pentyl-furylarylic acid (figure 3). Literature reports evidenced that malabaricone C to have AChE inhibitory activity of 44.0 µM and 1.94 µM while this study showed 5.75 µM of IC_{50} and the positive control donepezil hydrochloride showed IC_{50} of 0.072 µM. According to structure activity studies acetylated, benzoylated and crotonylated derivatives were inactive for AChE inhibitory activity.

Table 1. Anticholinesterase activity of the *M. fragrans* extracts

Crude extract	Percentage inhibition at the concentration of 100 µg/mL
n-Hexane	62.99
DCM	85.09
EtOAc	87.51
MeOH	96.75

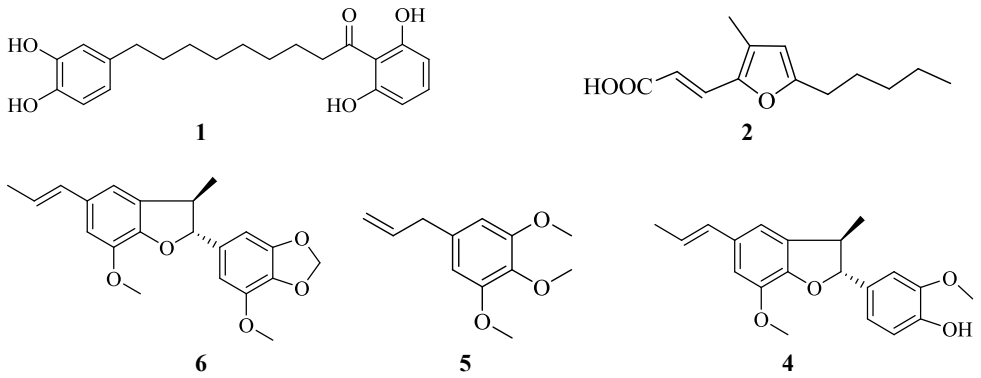

Figure 2. Chemical structures of compounds isolated from mace 1-6 (3 is a fatty acid)

Table 2. Acetylcholinesterase and *α*-glucosidase inhibitory activities and DPPH radical scavenging activity of compounds 1-6

Compound	Anticholinesterase activity		α-Glucosidase inhibitory activity		Antioxidant activity	
	Percentage inhibition[a)]	IC_{50} (µg/mL)	Percentage inhibition[a)]	IC_{50} (µg/mL)	Percentage inhibition[a)]	IC_{50} (µg/mL)
1	100.09[b)]	2.06±0.04	NA	NA	96.33	6.56±0.02
2	14.09	ND	90.63	51.02±0.01	27.98	213±0.08
3	27.72	300±0.09	89.69	46.74±0.01	NA	NA
4	48.26	111.3±0.07	NA	NA	73.34	24.74±0.09
5	NA	NA	30.60	ND	NA	NA
6	20.02	ND	NA	NA	NA	NA

[a)]assayed at 100 µg/mL; [b)] assayed at 12.5 µg/mL; ND - not determined; NA - not active; IC_{50} values of positive controls, donepezil HCl, acarbose and ascorbic acid, for anticholinesterase, *α*-glucosidase inhibitory and antioxidant activities were 0.03±0.00, 265.3±0.13 and 5.76±0.01 µg/mL, respectively

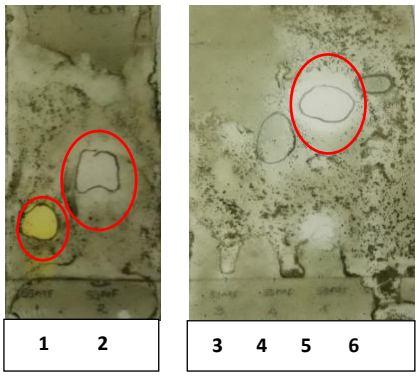

Figure 3. Qualitative analysis of antifungal activity of pure compounds of *M. fragrans* (**1-6**)

Conclusion

According to the results of AChE inhibitory assay, highest anticholinesterase activity was observed in EtOAc and MeOH extracts of *M. fragrans*. These two extracts furnished six compounds. Investigation of bioactivities of the pure compounds indicated that malabaricone C to have anticholinesterase and antioxidant activities, thus has a potential to be used for the treatment of AD. There have been evidences of intriguing connection between diabetes and AD. A fatty acid and 3'-methyl-5'-pentyl-furylarylic acid a furan fatty acid, scarcely found in plants showed high α-glucosidase inhibitory activity and are good source of anti-diabetic agents. Results of the antifungal activity of pure compounds indicated that malabaricone C, 3'-methyl-5'-pentyl-furylarylic acid and elemicin to have antifungal activity against *C. cladosporioides*. According to the results it could be concluded that the fruit aril of *M. fragrans* is a good source of anticholinesterase, antioxidant, α-glucosidase inhibitory and antifungal active compounds. Results of the structure activity relationship studies of malabaricone C indicated, that. hydroxyl groups present in malabaricone C are important for anticholinesterase activity.

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Selected References

Ellman, G. L., Courtney, K. D., Andres, V. J. and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology,* **7**, 88–95.

Sharp, H., Hollinshead, J., Bartholomew, B. B., Oben, J., Watson, A. and Nash, R. J. (2007). Inhibitory effects of *Cissus quadrangularis* L. derived components on lipase, amylase and α-glucosidase activity in vitro. *Natural Product Communications,* **2**, 817-822.

Tepe, B., Eminagaoglu, O., Akpulat, H. A. and Aydin, E. (2007). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts

of *Salvia verticillata* (L.) subsp. verticillata and *S. verticillata* (L.) subsp. Amasiaca (Freyn & Bornm.) Bornm. *Food Chemistry,* 100, 985–989.

Cuong, D., Hung, T. M., Han, H. Y., Roh, H. S., Seok, J. H., Lee, J. K., Jeong, J. Y., Choi, J. S., Kim, J. A. and Min, B. S. (2014). Potent acetylcholinesterase inhibitory compounds from *Myristica fragrans*. *Natural Product Communications,* 9, 499–502.

Publications

Sathya, S., Jayasinghe, L., Amarasinghe, N. R., Araya, H. and Fujimoto, Y. (2019). Enzyme inhibitors from the aril of *Myristica fragrans*, *South African Journal of Botany*, 130, 172-176.

Thesis Reference

Sathya, S. (2019). Investigation of Acetylcholinesterase Inhibitory Activity of Selected Sri Lankan Grown Spices as Potential Therapeutic Agents for Alzheimer's Disease. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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CHEMISTRY OF SECONDARY METABOLITES PRODUCED BY PLANT AND MARINE ENDOPHYTIC FUNGI AND BIOACTIVITY STUDIES

M. M. Qader *(PhD, MRSC), received his B.Sc. (1st Class Honours) in Chemistry from the Open University of Sri Lanka in 2013. He was attached to the Natural Products Research Group at the National Institute of Fundamental Studies while he was reading to his Ph.D. During his postgraduate studies he received the competitive Erasmus Mundas-GLink Fellowship to conduct part of his Ph.D. studies at the University of the West of Scotland, UK. Also he is the recipient of the Kandiah Award for Research from the Institute of Chemistry Ceylon in 2017. He completed his Ph.D. at the PGIS in 2019. At present, he is serving as a Natural Product Research Specialist at the University of Hawaii at Hilo, USA.*

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Accidental discovery of the broad spectrum antibiotic penicillin from the *Penicillium notatum* in 1928 was able to gain attention of the worldwide researches, chemist and microbiologist towards microbial natural products or microbial secondary metabolites. As a result, cholesterol lowering statins, anticancer, antiviral, antimicrobial, and immunomodulatory drugs which are clinically active at present were discovered. Therefore, the field of microbial natural products is believed to be one of the emerging sciences in drug discovery. These microbes are capable of adapting to different hosts in different habitats while maintaining a symbiotic relationship. In return microbes produced secondary metabolites (SM) which are beneficial to the host as a defensive shield. Among them entophytic fungi those are residing inside the plant tissues are the mostly studied organisms in the field. The previous studies concluded that the SMs produced by these microbes are structurally diverse and biologically active.

The study was mainly carried out to identify the bioactive SMs from Sri Lankan medicinal plants where less number of studies has been conducted and also its known that fungi from harsh conditions like marine habitats produced new and novel SMs where could be drugs or drug leads for drug discovery. The study was carried out with the objectives:

(I) To isolate entophytic fungi from Sri Lankan medicinal plants and from marine environment

(II) To identify the bioactive extracts by chemical profiling and bioassay screening

(III) To purify and isolate bioactive SMs from active extracts and assess their biological activity

Figure 1. Sri Lankan medicinal plants used for the study:(a) *Garcinia mangostana* (b) *Flacourtia inermis* (c) *Costus speciosus* (d) *Murraya koenigii*

Methodology

In this study, four Sri Lankan medicinal plants, *Costus speciosus, Flacourtia inermis, Garcinia mangostana* and *Murraya koenigii* (figure 1) were investigated for their endophytic fungi and the respective endophytes, *Bipolaris sorokiniana, Fusarium decemcellulare, Penicillium citrinum* and *P. verruculosum* were isolated and investigated for microbial SMs. Meanwhile, the fungi *Aspergillus sydowii, Alternaria alternata* and *Epicoccum* sp were isolated from a deep-sea marine animal from the Atlantic Sea, and from two sea grasses *Phragmites australis* and *Thalassia hemprichii* from the Red Sea respectively, were investigated. Fungal strains were identified by morphological and molecular means. The isolated fungi were fermented for 28 days in nutrient media and for marine fungi, sea salt was incorporated. The microbial SMs were extracted into organic solvents of different polarity. The crude extracts from plant–endophytic fungi were screened for *Cladosporium cladosporioides* antifungal activity, DPPH radical scavenging activity, brine shrimp cytotoxicity, lettuce seed phytotoxicity and α–amylase enzyme inhibitory activity. The chromatographic separation of selected crude extracts with good bioactivities over silica gel, Sephadex LH–20, PTLC and RP–HPLC were employed. Structure characterization of the microbial SMs was done by extensive analysis of 1D and 2D NMR together with HRMS data.

Results and Discussion

This is the first report of the isolation of respective plant endophytic fungi from their respective hosts. The crude extracts of the fungal strains isolated from medicinal plants showed interesting bioactivities, which encouraged us to do the further studies. The crude extracts of *B. sorokiniana* showed strong phytotoxicity and antifungal activity; strong DPPH radical scavenging and brine shrimp toxicity showed by *P. citrinum* and *P. verruculosum*; and strong anti-aging, anti-microbial, anti-biofilm and cytotoxicity activity for the extracts of the marine endophytic fungi *A. alternata, A. sydowii* and *Epicoccum* sp. Further purification of bioactive extracts over different chromatographic techniques, and extensive analysis of 1D and 2D NMR spectral data together with HRMS data, special dereplication databases (AntiBase, AntiMarine, Dictionary of Natural Products, Reaxys and SciFinder) and the literature data enable us to identify bioactive microbial SMs: helminthosporol and helminthosporal acid from *B. sorokiniana*, shikimic acid from *F. decemcellulare*, GKK1032B and citrinin from *P. citrinum* and vermistatin from *P. verruculosum*. 3,5–sydotriazinedione, (*R*)–sydosine, brefeldin A and phenamide from *A. sydowii*, tenuazonic acid, analog of oxydiketopiperazine, analog of trioxobutanamide,2,5–dimethyl–7–hydroxychromone, altenusine, alterariol, alternariol monomethyl ether, alterotoxin I, alterotoxin II and alteichin from *A. alternata* and cyclo–(L–pro–L–val), cyclo–(L–pro–L–tyr), cyclo–(L–ile–L–pro), cyclo–(L–*allo*–ile–L–pro), cyclo–(L–phe–L–pro), cyclo–(L–trp–L–pro–L–phe) and N–(2–phenylethyl)–acetamide from *Epicoccum* species. Mosher ester analysis and advanced Marfey's analysis were used to establish the absolute configuration of the new microbial SMs identified from this study. Strong phytotoxic and antifungal activities were displayed by helminthosporol and helminthosporal acid respectively. The production of high quantity of citrinin from *P. citrinum,* species level identification of the source of the complex polyketide–amino acid hybrid compound, GKK1032B were reported for the first time. Furthermore, from marine fungi, six new natural compounds (3,5–sydotriazinedione, (*R*)–sydosine**,** analog of oxydiketopiperazine, analog of trioxobutanamide, cyclo–(L–*allo*–ile–L–pro) and cyclo–(L–trp–L–pro–L–phe) were isolated.

Conclusion

This study emphasizes the importance of endophytic fungi isolated from different habitats which are able to produce structurally diverse and biologically active microbial metabolites. Therefore endophytes are now recognized as a potential source for agriculture, bioindustry and medicine.

Source of Funding

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Selected References

Cragg, G.M. and Newman, D.J. (2005). Biodiversity: A continuing source of navel drug leads. *Pure and Applied Chemistry,* **77**, 7–24.

Debbab, A., Aly, A. H., Lin, W. H. and Proksch, P. (2010). Bioactive compounds from marine bacteria and fungi. *Microbial Biotechnology,* 3, 544–563.

Gunatilaka, A. L. and Wijeratne, E. K. (2000). Natural products from bacteria and fungi. *Encyclopedia of Life Support Systems (EOLSS).*

Publications

Qader, M.M., Kumar, N., Jayasinghe, L. and Fujimoto, Y. (2015). Production of antitumor antibiotic GKK1032B by *Penicillium citrinum*, an endophytic fungus isolated from *Garcinia mangostana* fruits. *Med Aromat Plants,* 5, 225.

Qader, M.M., Kumar, N.S., Jayasinghe, L., Araya, H. and Fujimoto, Y. (2017). Bioactive sesquiterpenes from an endophytic fungus *Bipolaris sorokiniana* isolated from a popular medicinal plant *Costus speciosus*. *Mycology,* 8, 17-20.

Qader, M.M., Kumar, N.S., Jayasinghe, L. and Fujimoto, Y. (2018). Shikimic acid production by *Fusarium decemcellulare*, an endophytic fungus isolated from *Flacourtia inermis* fruits. *Journal of Biologically Active Products from Nature* 8, 43-50.

Soldatou, S., Qader, M.M., Pavesi, C., Miranda, K.J., Diyaolou, O.A., Hamed, A.A., Rateb, M.E. and Ebel, R. (2020). Egyptian fungal antibiotic metabolites-from pharaohs to modern textiles. *Marine Drugs,* 18, 59.

Thesis Reference

Qader, M.M. (2019). Chemistry of Secondary Metabolites Produced by Plant and Marine Endophytic Fungi and Bioactivity Studies. Ph.D. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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REAL TIME TRAFFIC CONTROL OPTIMUM PHASES AT ROAD ISOLATED INTERSECTIONS AND ARTERIAL NETWORKS

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Road transportation network is significant backbone of current society with the increasing demand of mobility. Due to the increasing number of vehicles, traffic congestion has become a serious problem in big cities. The traffic signal setting problem is to investigate how to set the given traffic signals such that the total waiting time of vehicles on the roads is minimized. The advantages of proper traffic signal control are to move traffic in an orderly fashion, minimize delay to vehicles and pedestrians, reduce crash-producing conflicts, and maximize vehicle capacity for each intersection approach.

This work presents four major non-linear programming models. The first and second models are non-linear programming models to minimize the aggregate delay time of vehicles and the number of vehicles on each lane by optimizing the total number of vehicles at the signalized single intersection and two intersections arterial network respectively, under fixed incoming flow rates of vehicles according to maximum cycle time. The third and fourth models are non-linear programming models to minimize the aggregate delay time of vehicles and the number of vehicles on each lane by optimizing the total number of vehicles at the signalized single intersection and two intersections arterial network respectively, under time-varying (inter green-red-green signal transition time) incoming flow rates of vehicles. The first and third models are modeled under special oversaturation condition 1 (Case 1) and second and fourth models are modeled under special oversaturation condition 2 (Case 2).

This research addresses real-time traffic congested problem at a road isolated intersection and a two intersections road arterial network. The objectives of this work are
(I) to reduce congestion and

(II) to minimize the delay time of vehicles

by minimizing the number of waiting vehicles and aggregate delay time of vehicles at a road isolated intersection and a two intersections road arterial.

Methodology

Four main methodologies are developed to achieve our goal. The four main methodologies are:

1. Two real-time non-linear programming models are developed to optimize signal control at an isolated road intersection and two intersections arterial network respectively by considering special oversaturation condition 1 with some initial signal timing control conditions and incoming flow rates of vehicles according to maximum cycle time.
2. Two real-time non-linear programming models are developed to optimize signal control at an isolated road intersection and two intersections arterial network respectively by considering special oversaturation condition 2 with some initial signal timing control conditions and incoming flow rates of vehicles according to maximum cycle time.
3. Two real-time non-linear programming models are developed to optimize signal control at an isolated road intersection and two intersections arterial network respectively by considering special oversaturation condition 1 with some initial signal timing control conditions and incoming flow rates of vehicles according to time varying (inter green-red-green signal transition time rather than maximum cycle time).
4. Two real-time non-linear programming models are developed to optimize signal control at an isolated road intersection and two intersections arterial network respectively by considering special oversaturation condition 2 with some initial signal timing control conditions and incoming flow rates of vehicles according to time-varying (inter green-red-green signal transition time rather than maximum cycle time).

The models of above-mentioned methodologies calculate the green signal time of signals to minimize the waiting number of vehicles and aggregate delay time of vehicles by optimizing the total number of vehicles at an isolated intersection and an arterial network. The real-time data of the number of vehicles is counted using video cameras installed at the road intersection and at arterial network.

Results and Discussion

Isolated Intersection (Model 1)

Simulation results are shown in the figure 1 and figure 2:

From camera readings: $N_1(1) = 16$, $N_2(1) = 10$, $N_3(1) = 18$, $N_4(1) = 12$. Phase sequence order (signal order): Lane 3 signal, Lane 1 signal, Lane 4 signal, Lane 2 signal (Corresponds to decreasing order of number of vehicles on lanes).

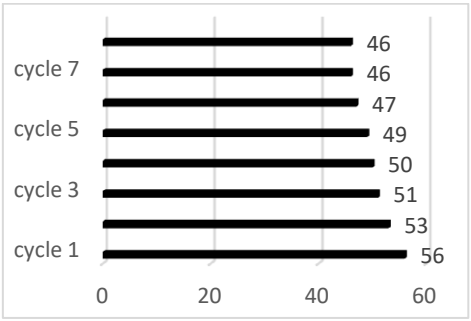

Figure 1. Total number of vehicles (decreases from cycle to cycle) for Model 1 in each cycle on Lane 1, Lane 2, Lane 3 and Lane 4 of the intersection

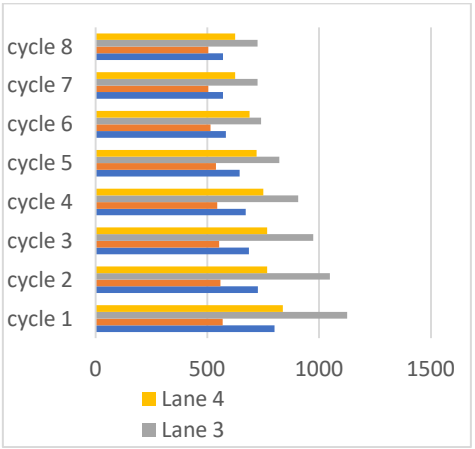

Figure 2. Aggregate delay time of vehicles (decreases from cycle to cycle) for Model 1 on Lane 1, Lane 2, Lane 3 and Lane 4 of each cycle

Arterial Networks (Model 4)

Simulation results are shown in the figure 3, figure 4, figure 5 and figure 6:

From camera readings: $N_{11}(1) = 26, N_{12}(1) = 20, N_{13}(1) = 25,\ N_{14}(1) = 15, N_{21}(1) = 26, N_{22}(1) = 17, N_{23}(1) = 28,\ N_{24}(1) = 21.$

Phase sequence order (signal order):
Intersection 1: Lane 12 signal, Lane 14 signal, Lane 11 signal, Lane 13 signal.
Intersection 2: Lane 22 signal, Lane 24 signal, Lane 21 signal, Lane 23 signal.

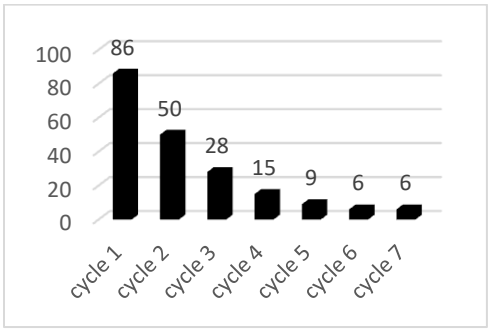

Figure 3. Total number of vehicles (decreases from cycle to cycle) for Model 4 in each cycle for Intersection 1 of arterial network

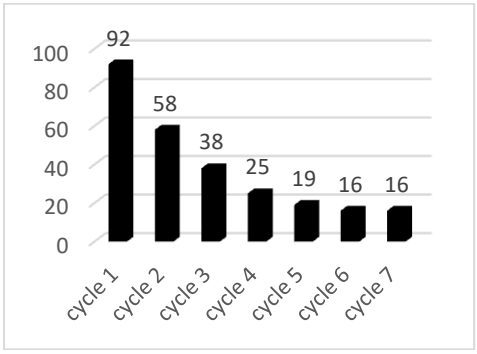

Figure 4. Total number of vehicles (decreases from cycle to cycle) for Model 4 in each cycle for Intersection 2 of arterial network

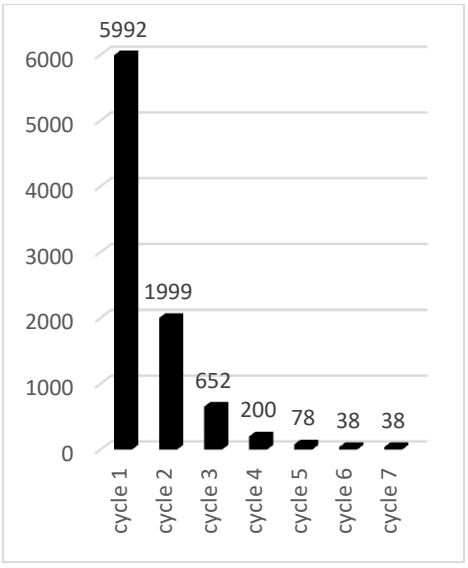

Figure 5. Aggregate delay time (decreases from cycle to cycle) for Model 4 in each cycle for Intersection 1 of arterial network

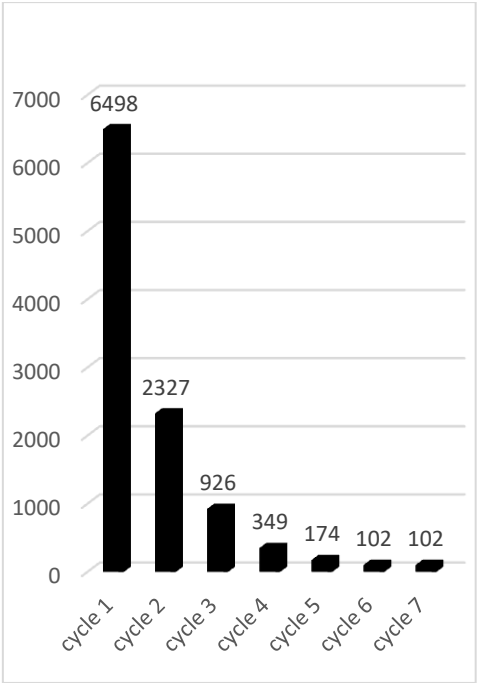

Figure 6. Aggregate delay time (decreases from cycle to cycle) for Model 4 in each cycle for Intersection 2 of arterial network

Conclusion

Road transportation network is significant backbone of current society with the increasing demand of mobility. Due to the number of vehicles rapidly increases, traffic congestion has become a serious problem in big cities. The results clearly show that the number of waiting vehicles and aggregate delay time of vehicles on each lane decrease from cycle to cycle. The advantages of this proper traffic signal control are to move traffic in an orderly fashion, minimize delay to vehicles and pedestrians, reduce crash-producing conflicts, and maximize vehicle capacity for each intersection approach.

Selected References

Allsop, R.E. (1971). Delay-minimizing settings for fixed-time traffic signals at a single road junction. *IMA Journal of Applied Mathematics,* 8 (2), 164–185.

Bell, M.G.H. (1992). Future directions in traffic signal control. *Transportation Research,* 26 (4), 303–313.

Branch, M.A., Coleman, T.F. and Li, Y. (1999), A Subspace, Interior, and Conjugate Gradient Method for Large-Scale Bound-Constrained Minimization Problems. *SIAM Journal on Scientific Computing*, 21, Number 1, pp. 1-23.

Fletcher R. (1987). Practical Methods of Optimization. *John Wiley and Sons*.

Gartner, N. H., Pooran, F. J., and Andrews C. M. (2002). Optimized Policies for Adaptive Control Strategy in Real-Time Traffic Adaptive Control Systems. *Transportation Research Record,* 1811, pp. 148-156.

Chen, P., Liu,H., Qi, H. S., and Wang, F. J. (2013). Analysis of delay variability at isolated signalized intersections. *Journal of Zhejiang University*-SCIENCE A (Applied Physics & Engineering), 14(10), 691-704.

Publications

Raviraj, Y. and Daundasekera. W.B. (2018). Real Time Traffic Control to Optimize Waiting Time of Vehicles at A Road Intersection. *International Journal of Research in Engineering and Science (IJRES)*, 06, no. 04, pp. 25–33.

Raviraj, Y. and Daundasekera. W.B. (2018). Real Time Traffic Control to Optimize Waiting Time of Vehicles at Road Arterial Networks. *International Journal of Engineering Science Invention (IJESI)*, 07, no. 07, pp. 48-58.

Raviraj, Y. and Daundasekera. W.B. (2019). Real Time Traffic Control Under Time Varying Incoming Flow Rates of Vehicles to Optimize Waiting Time of Vehicles at Road Intersection. *International Journal of Engineering, Science and Mathematics (IJESM),* 08, issue. 07, pp. 50-63.

Raviraj, Y. and Daundasekera. W.B. (2019). Real Time Traffic Control Under Time Varying Incoming Flow Rates of Vehicles to Optimize Waiting Time of Vehicles at Road Arterial Network. *International Journal of Applied Science and Technology (IJAST)*, 09, no. 04, December, pp. 24-43.

Thesis Reference

Raviraj, Y. (2019). Real Time Traffic Control Optimum Phases at Road Isolated Intersections and Arterial Networks. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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BEHAVIOUR OF SINGLE BUBBLE SONOLUMINESCENCE IN DIFFERENT LIQUID MEDIA

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Sonoluminescence (SL) is a phenomenon of periodic light emission from a tiny bubble or bubble cloud trapped on a standing ultrasonic field in a liquid. Sonoluminescence requires the cavitation process for its existence although not all process of cavitation can lead to sonoluminescence. There are two types of sonoluminescence; Multi bubble sonoluminescence (MBSL) and Single bubble sonoluminescence (SBSL). There has been a large number of theoretical and experimental investigations carried out to explore and understand the extreme conditions which occurs at the last stage of the bubble collapse and to predict the exotic physical conditions which take place inside the SL bubbles at the time of light emission.

As the bubble collapses, vibrational energy produced by transducers gets concentrated by at least a factor of 4×10^{11} to produce flashes of light in the ultraviolet (UV) range. These flashes of UV light have durations much shorter than a nanosecond. At the last stage of the bubble collapse, the temperature inside the bubble reaches astonishingly over 15000 K while pressure inside the bubble approaches 6000 atm. Also the bubble wall reaches acceleration over 10^{11} g near the maximum implosion. SBSL is observed by only with the bubbles having ambient radii between 1 nm to 10 nm and during the collapse radii of these bubbles come down to 0.1 nm.

Moshaii *et al.* (2011), theoretically predicted that temperature dependence of light emission in sulfuric acid is quite opposite to the same observed in water. Through computational simulations, they have found that in fact light intensity decreases as the temperature of sulfuric acid in 85 wt% solution decreases. This remarkable result has never been tested experimentally. In this research the temperature dependence of the light emission in sulfuric acid was experimentally investigated and the results found by Moshaii *et al.* (2011) were confirmed. The study was carried out with the following objectives:

(I) To test predictions made on the temperature dependence of SBSL in sulfuric acid and

study similarities between SBSL in sulfuric acid and selenic acid

(II) To investigate the UV portion of SBSL spectrum in water using fluorescent sodium solution

(III) To study the SBSL in 98 wt% sulfuric acid and other concentrations (85 wt%, 65 wt%, 58 wt% and 40 wt%) and find the concentration for which SBSL emission may become independent of the temperature

Methodology

The SBSL experimental setup consists of a spherical acoustic resonator, an ultrasonic generating system and a data acquisition (DAQ) system. The acoustic resonator system is driven harmonically at the fundamental resonance frequency of the flask, which is about 25.0-27.5 kHz. The ultrasonic sound generation part was constructed with a function generator (Agilent 33220A), a power amplifier and two pairs of hollow, right circular cylindrical piezoelectric transducers glued opposite sides to the outer surface of the flask symmetrically. During the experiment, a photomultiplier tube (PMT) was used to detect the SBSL bubble and measure the relative intensity of the light pulse. Signals from PMT were fed into a computer via a data acquisition board (PCI-DAS4020/12), and then they were monitored and recorded with TraceDAQ data acquisition software for post action analysis. Later, the photon count was determined by software developed in our laboratory using the dark reading as the reference. The SBSL spectrum is obtained using an Ocean Optics QE65000 Pro fiber-based with a homemade lens-collimator system for collecting light from the bubble. The entire system is calibrated in the 220-920 nm range with an Ocean Optics DH-2000-CAL NIST-traceable fiber-based calibration source. Then, SBSL was obtained for different host liquids.

Results and Discussion

Temperature dependence of SBSL in sulfuric acid

Based on the results of the experiments as shown in figure 1, it was found that the intensity increases as temperature increases from 15 °C and 25 °C, confirming what has been predicted by Moshaii *et al*. (2011) theoretically. This behavior is completely different from what has been observed for SBSL in water. Beyond 25 °C, SBSL intensity in 85 wt % sulfuric acid solutions became temperature independent.

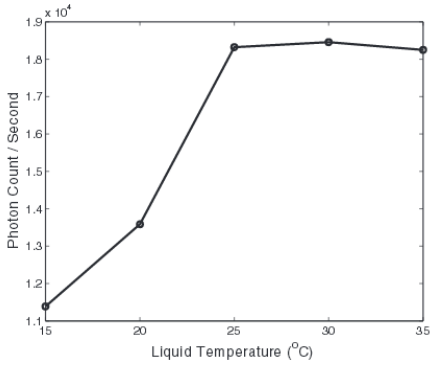

Figure 1. Number of photons detected per second by the PMT for five different temperatures for SBSL in 85 wt% sulfuric acid

Further, it was observed that as the temperature increases, more UV photons and less visible photons contribute to the total intensity, indicating higher bubble temperatures at higher temperatures as shown in figure 2. when the driving acoustic pressure is larger than a threshold value, it was possible to observe the fragmentation of the bubble into a few multiple bubbles which move away from the center of the resonator towards the flask walls, producing MBSL.

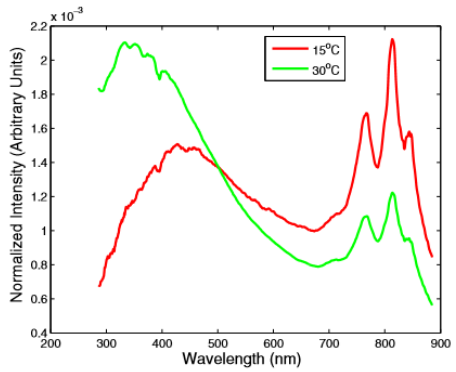

Figure 2. SBSL spectra of 85 wt% sulfuric acid for 15 °C and 30 °C. Ar emission lines are most prominent at 15 °C

As a result, unlike SBSL in water, the intensity threshold for sulfuric acid at each temperature is not determined by the stability conditions (shape or positional) but by the driving pressure at which the transition from SBSL to MBSL takes place.

Behavior of SBSL in Selenic Acid, Sulfuric Acid and Water

Selenic acid shows intermediate behavior compared with sulfuric acid and water. It is possible to obtain very stable SBSL in selenic acid as same as SBSL in water although it produced very high visible light intensity comparable with sulfuric acid when the sound field is increased. At the low degassed level, it produces M-SBSL and with the increment of the pressure it produces MBSL which is almost similar to SL in sulfuric acid. Expected emission lines from selenic acid were conspicuously absent, suggesting a different mechanism for light production in SBSL and the spectrum for SBSL bubbles in water is consistent with that previously reported. The radiated energy is not a high value compared with sulfuric acid, and the peak of the spectrum appears to shift towards longer wavelengths for the selenic acid in higher transducer voltages.

Investigation of the UV portion of SBSL spectrum in water using fluorescent sodium solution

The temperature dependence of the SBSL light intensity in water and dilute fluorescein sodium solutions was investigated. Stable SBSL is observed in fluorescein sodium solution with higher intensity compared to water. For both water and fluorescein sodium solutions, according to the photon count, the maximum intensity of the SBSL bubble is observed at the lowest ambient temperature.

Concentration dependence of SBSL in sulfuric acid

Spectral lines appeared for higher concentrations, 98 wt% and 85 wt% for the whole temperature range while they are more prominent at lower temperatures on 98 wt% sulfuric acid . However, the spectral lines appeared for concentrations of 58 wt% to 65 wt% only for the lower temperatures. Further, the peak value of the spectrum for the concentration greater than 50 wt% is shifted towards the lower wavelength range (i.e. UV) when the temperature is increased indicating the UV contribution of the spectrum is higher. As evident from the figure 3, the maximum SL radiation is observed at 30 °C from the solution of around 65 wt% sulfuric acid and it is in agreement with the experimental results published by Moshaii *et al.* (2011).

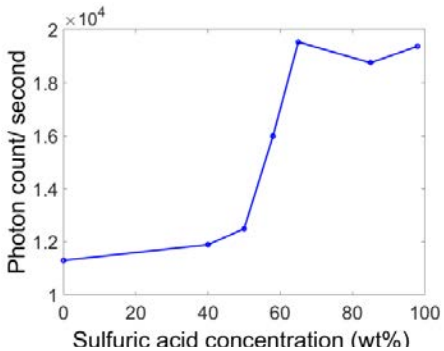

Figure 3. Maximum SBSL intensity obtained from different sulfuric acid solutions (0 to 98 wt%). SBSL in deionized water was considered as 0 wt% sulfuric acid

Moreover, the peak value of the spectrum for the concentration less than 50 wt% is shifted towards the higher wavelength range when the temperature increases. However, the maximum SL radiation is observed from the solution of 98 wt% of H_2SO_4 for the entire temperature and concentration range.

Conclusions

Based on the results of the experiments in this study, it was possible to confirm the theoretical predictions made by Moshaii *et al.* (2011) regarding the temperature dependence of SBSL intensity in 85 wt% sulfuric acid solutions. It is possible to conclude that unlike SBSL in water, the intensity threshold at each temperature is not determined by the stability conditions but by the driving pressure at which the transition from SBSL to MBSL takes place.

It is possible to predict that the temperature independence concentration can be expected in the region between 58-65 wt% although temperature independence concentration was not directly obtained under this experiment. However, 98 wt% sulfuric acid shows the highest SL radiation for entire temperature and concentration range.

It was found that by adding fluorescein sodium, the intensity of the light in water got increased indicating that considerable amount of UV light is absorbed by water in the flask.

Selected References

Dan, M., Cheeke, J.D.N. and Kondic, L. (2000). Dependence of single bubble sonoluminescence on ambient pressure. *Ultrasonic,* 38, 566.

Kappus, B., Khalid, S., Chakravarty, A. and Putterman, S. (2011a). Phase transition to an opaque plasma in a sonoluminescing bubble. *Physical Review Letters* 106, 234302.

Moshaii, A., Imani, K.H. and Silatani, M. (2009). Sonoluminescence radiation from different concentrations of sulfuric acid. *Physical Review E* 80, 046325.

Moshaii, A., Nezhad, S.T. and Faraji, M. (2011). Temperature dependency of single bubble sonoluminescence in sulfuric acid. *Physical Review E* 84, 046301.

Publications

Herath, P and Nanayakkara, A. (2013). Investigation of Light Intensity of Single Bubble Sonoluminescence (SBSL) in Water and Fluorescein Sodium Solution. *Proceedings of the Technical Sessions*, 29 43 IPSL.

P.S. Herath and A.Nanayakkara (2014) Behavior of Single Bubble Sonoluminescence in Selenic Acid, Sulfuric Acid and water. *Proceedings of the Postgraduate Institute of Science Research Congress*, Sri Lanka.

Bandara, V. Herath, P. and Nanayakkara, A. (2015) Temperature dependence of single-bubble Sonoluminescence threshold in sulfuric acid: An experimental study. *Physical Review E* 91.

Thesis Reference

Herath, H.M.P.S. (2019). Behaviour of Single Bubble Sonoluminescence in Different Liquid Media. M.Phil. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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INVESTIGATION OF SPIN REORIENTATION OF FERROMAGNETIC THIN FILMS USING MODIFIED THIRD ORDER PERTURBED HEISENBERG HAMILTONIAN

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In recent years it has become possible to grow high quality ferromagnetic thin film materials on non- magnetic substrates experimentally. These structures showed new properties that were not seen previously and had many applications in the modern information age. A lot of research is directed at data storage in thin film magnetic media with the ever-increasing demand for data storage in high capacities. Thin film magnetic media is produced by coating a nanometer layer of a magnetic compound on a substrate. The density of data storage, the speed of writing and reading data all depend on the physical properties of this layer. For example, there is an ideal thickness of the magnetic layer and an ideal crystallographic orientation of the coating that maximizes data density and optimizes reading and writing time. In order to determine such physical parameters of the magnetic coating, experiments should be performed with various film thicknesses etc. Therefore, the Heisenberg Hamiltonian was used to obtain a theoretical insight to determine properties of magnetic thin films.

The current project addresses the 3^{rd} order perturbed Heisenberg Hamiltonian with all seven magnetic energy parameters which was solved for simple cubic (sc), face centred cubic (fcc) and body centred cubic (bcc) structured ferromagnetic films with spin layers up to 100 by a MATLAB computer program for the first time.

The study was carried out with the following objectives:

(I) To identify the variation of magnetic easy axis and the total magnetic energy with number of layers

(II) To identify the variation of easy and hard directions with the number of spin layers

(III) To investigate the easy axis orientation of sc, fcc and bcc ferromagnetic thin films

Methodology

The Heisenberg Hamiltonian of ferromagnetic films can be written as

$$H= \frac{J}{2}\sum_{m,n}\vec{S}_m.\vec{S}_n+\frac{\omega}{2}\sum_{m\neq n}\left(\frac{\vec{S}_m.\vec{S}_n}{r_{mn}^{3}}-\frac{3(\vec{S}_m.\vec{r}_{mn})(\vec{r}_{mn}.\vec{S}_n)}{r_{mn}^{5}}\right)-\sum_m D_{\lambda_m}^{(2)}(S_m^{z})^2-\sum_m D_{\lambda_m}^{(4)}(S_m^{z})^4$$

$$-\sum_{m,n}[\vec{H}-(N_d\vec{S}_n/\mu_0)].\vec{S}_m-\sum_m K_s Sin2\theta_m$$

Here N, m (or n), J, $Z_{|m-n|}, \omega, \Phi_{|m-n|}, \theta_m(\theta_n), D_m^{(2)}, D_m^{(4)}, H_{in}$, H_{out}, N_d and K_s are total number of layers, layer index, spin exchange interaction, number of nearest spin neighbors, strength of long range dipole interaction, partial summations of dipole interactions, azimuthal angles of spins, second and fourth order anisotropy constants, in plane and out of plane applied magnetic fields, demagnetization factor and stress induced anisotropy constants, respectively.

By plotting a 3-D plot of energy versus angle and stress induced anisotropy, the values of stress induced anisotropy corresponding to energy minimums and maximums were determined. By plotting the graphs of energy versus angle at these different stress induced anisotropy values, the easy and hard directions were determined. Similar graphs were plotted for the other six magnetic to determine the easy and hard directions. Graphs of energy versus angle will be plotted by keeping all the magnetic energy parameters at constant values for each number of spin layers to determine the variation of magnetic easy directions, hard direction and corresponding energies with the number of spin layers. After that variation of easy and hard directions with the number of spin layers will be investigated for sc, fcc and bcc ferromagnetic thin films. Finally, the average out of plane spin component will be found as a function of temperature.

Results and Discussion

Magnetic energy variation of sc, fcc and bcc ferromagnetic thin films with seven magnetic parameters

Number of minimums and maximums are proportional to number of spin layers in 3-D plots up to 100 spin layers. Also, energy $\frac{E(\theta)}{\omega}$ is increased with the number of spin layers as shown in figure 1. Minimums and maximums are closely packed with their spin numbers. This may be related to the fact that $\frac{N_d}{\mu_0\omega}$ contributes only a constant to the equation of total energy.

Energies of the graphs increase with number of spin layers in the study of stress induced anisotropy. Because the total number of spins in film increases with number of spin layers, the energy of a film with more spin layers must be simply higher. For simple cubic ferromagnetic films with 70, 80 and 90 spin layers, the magnetic easy and hard directions are independent of the value of $\frac{J}{\omega}$. Second and fourth order anisotropy also contributed to the magnetic energy variations of sc, fcc and bcc ferromagnetic thin films. When N=50 and N=60, magnetic easy and hard directions do not depend on fourth order anisotropy in simple cubic ferromagnetic films. In simple cubic with 60 spin layers magnetic easy and hard directions are independent of second order anisotropy. Also, in fcc thin films with 90 layers, easy and hard directions are independent of fourth order anisotropy.

Angle between easy and hard directions is 90 degrees in the case of energy variation with out of plane magnetic field for simple cubic three spin layered films.

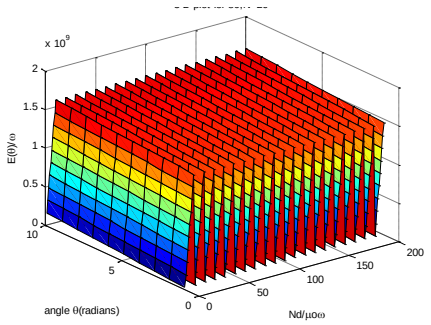

Figure 1. 3-D plot of $\frac{E(\theta)}{\omega}$ versus angle and $\frac{N_d}{\mu_0\omega}$ (sc, N=20)

Variation of magnetic easy directions

In simple cubic ferromagnetic thin films the magnetic easy axis rotates from out of plane to in plane direction of the film. Magnetic anisotropy energy gradually increases from 548.10 to 3631, as the number of spin layers is increased from 10 to 100. However, the angle between magnetic easy and hard axis does not

change considerably. Similarly, as the number of spin layers is increased in face centered cubic thin films, the magnetic easy axis rotates from out of plane to in plane direction.
Also, in bcc ferromagnetic thin films, the magnetic easy axis gradually rotates from perpendicular to in plane direction of the film plane. The negative value of energy along easy direction gradually increases with the number of spin layers. However, the angle between easy and hard directions changes slightly.

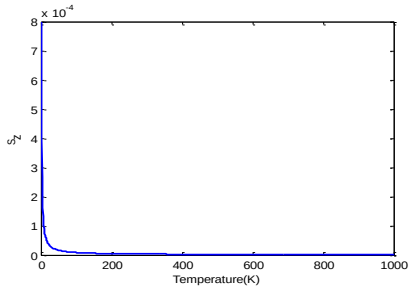

Figure 2. Graph of $\overline{S_z}$ versus temperature for fcc *N*=11 layers. The graph indicates a strong in plane orientation above a particular temperature of 484 K (211 ^{0}C)

Easy axis orientation of sc, fcc and bcc ferromagnetic thin films
According to the theoretical model, the in-plane orientation temperature for fcc structured ferromagnetic films with 11 layers is 484 K as shown in figure 2. This value agrees well with experimental data for CoPt/AlN films fabricated on fused quartz substrates by some other researchers. The average value of out of plane spin component at the spin reorientation temperature decreased to 1.6% of its initial value. This implied that the easy axis orients in the plane of the film above 484 K.

According to the figure 3, the in-plane orientation temperature for fcc structured ferromagnetic films with 27 layers is 305 K. This value well agrees with experimental data for sputtered fcc structured Gd thin films grown on Si (100) substrates.

In addition, the spin reorientation temperature is highly sensitive to the stress induced anisotropy and demagnetization factor. For sc thin films with 100 layers, 571 K is the spin reorientation temperature. The stress induced anisotropy plays a significant role in soft magnetic materials according to some experimental studies. A strong in plane orientation can be seen above temperature of 342 K (69 ^{0}C) for bcc thin films with 100 layers.

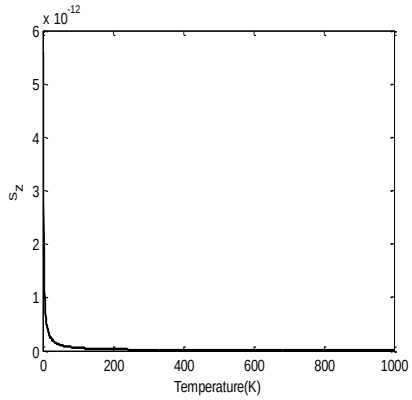

Figure 3. Graph of $\overline{S_z}$ versus temperature for fcc *N*=27 layers. In this graph, below 305 K most of the spins are in the out of plane direction, and beyond 305 K most of the spins are in the in-plane orientation

Conclusions
In simple cubic ferromagnetic thin films and fcc thin films the magnetic easy axis rotates from out of plane to in plane direction of the film. Also, in bcc ferromagnetic thin films, the magnetic easy axis gradually rotates from perpendicular to in plane direction of the film plane, as the number of spin layers is increased. Theoretical data in this research quantitatively agree with the experimental data obtained for ferromagnetic Ni and Fe thin films by some other researchers. The in-plane orientation temperature for fcc structured ferromagnetic films with 11 layers is 484 K. This value well agrees with experimental data for CoPt/AlN films fabricated on fused quartz substrates by some other researchers. Also, the stress induced anisotropy plays a significant role in soft magnetic materials according to some experimental studies. A strong in plane orientation can be seen in the above temperature of 342 K (69 ^{0}C) for bcc thin films with 100 layers.

Selected References
Samarasekara, P. and Saparamadu, U. (2013). Easy axis orientation of Barium hexa- ferrite films as explained by spin reorientation. *GESJ: Physics*, 1 (9).

Lederman, D., Ramirez, R. and Kiwi, M. (2004). Monte Carlo simulations of exchange

bias of ferromagnetic thin films on FeF2(110). *Phys. Rev*., B (70), 18442.

Samarasekara, P. and Rajakaruna, P.(2013), Orientation of easy axis of ferromagnetic films as explained by third order perturbed Heisenberg Hamiltonian. *Research & Reviews: Journal of Physics*, 2 (3), 5-9.

Bertelli, T. P., Passamani, E. C., Larica, C., Nascimento, V.P., Takeuchi, A. Y., Pessoa, M.S., (2015). Ferromagnetic properties of fcc Gd thin films. *Journal of Applied Physics*, 117, 203904.

Publications

Samarasekara, P. and Yapa, N.U.S. (2016). Third order perturbed Heisenberg Hamiltonian of bcc ferromagnetic ultra-thin films. *Research & Reviews: Journal of Physics-STM journals*, 5(2), 23-30.

Samarasekara, P. and Yapa, N.U.S. (2016). Third order perturbed energy of fcc ferromagnetic thin films as described by Heisenberg Hamiltonian. *Ceylon Journal of Science*, 45 (2), 71-77.

Samarasekara, P. and Yapa, N.U.S. (2016). Simple cubic ferromagnetic films with three layers as described by third order perturbed Hamiltonian. *GESJ: Physics*, 2 (16), 3- 11.

Yapa, N.U. S., Samarasekara, P. and Dehipawala, S. (2017). Variation of magnetic easy direction of bcc structured ferromagnetic films up to thirty spin layers. *Ceylon Journal of Science*. 46 (3), 93-103.

Samarasekara, P. and Yapa, N.U.S. and Fernando, C.A.N. (2018). Third order perturbed modified Heisenberg Hamiltonian of fcc structured ferromagnetic films with seventy spin layers. *GESJ: Physics*, no.1 (18).

Samarasekara, P. and Yapa, N.U.S. (2017). Third order perturbed Heisenberg Hamiltonian of sc ferromagnetic films with fifty spin layers. *GESJ: Physics 2* (18), pp. 64-72.

Yapa, N.U.S. and Samarasekara, P. (2016). Study of non-oriented ultra-thin ferromagnetic thin films. *PGIS Research Congress*.

Thesis Reference

Yapa, N.U.S. (2019). Investigation of Spin Reorientation of Ferromagnetic Thin Films Using Modified Third Order Perturbed Heisenberg Hamiltonian. Ph.D. Thesis, Postgraduate Institute of Science, University of Peradeniya.

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Sri Lanka

AGENT COMPATIBLE ITEM RESPONSE THEORY MODEL AND A CLUSTERING MECHANISM FOR E - LEARNING

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It is observed that the North Central Province has very low pass rate in national level examinations, especially in the General Certificate in Education (Ordinary Level). Therefore, it is a timely requirement to find reasons for this situation and to propose suitable remedies for it. After conducting several surveys by targeting primary level students, it is revealed that a primary reason for the low - pass rate in Mathematics was the lack of knowledge in Mathematical concepts and the lack of primary teachers. Therefore, an Agent Based E Learning System is expected to be introduced to eradicate this situation. In order to develop such a system, it is required to develop a theoretical background for the Agent Based E Learning system. Thus, the primary objective of this research is to develop the theoretical background for the Agent Based E Learning.

The proposed system, before presenting any lesson, automatically identifies the student's competency level by using his psychometric measurements and clusters the student for the appropriate competency level. To achieve above two requirements, a new time based model for Item Response Theory (IRT) was developed. Then, to cluster students according to their psychometric measurements, a new unsupervised clustering mechanism was developed. The new two phase unsupervised clustering algorithm and the new IRT model were successfully tested with real data sets. A suitable web framework is also designed for the proposed Agent Based E Learning System. Therefore, the main theoretical background concepts for the Agent Based E Learning System were developed and tested during this research. A student's response to a question always depends on two factors. First, the ability level of the examinee (student) and the difficulty level of the question (item). In most of the traditional examinations, only the ability is tested. In more advanced examinations such as Test of English as a Foreign Language (TOEFL), the difficulty level of the questions, which is calculated based on the responses of all examinees, also taken into consideration in the evaluation process. However, as in online testing, the response time to a particular question also can be recorded, it opens another dimensionality to the evaluation process. Maris (1993) suggested to use Gamma Distribution for Response Time (RT)

modelling for evaluation processes. In this research, a novel mechanism to adapt the response time into the existing Rasch model for evaluation process is proposed and evaluated.

Methodology

Response Time and the speed

The response time (RT) and the speed are not in traditional latent variable models. Thus, it is required to consider other extra parameters also. A main extra parameter is labor intensity. It is related in the same fundamental way as the probability of success, the ability and the difficulty in the Rasch model shown in figure 1.

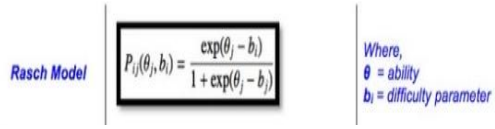

Figure 1. Rasch model

Proposed modification to the Rasch model

In order to develop new IRT model, the speed of a student j is considered for time (T) for item i;

$$\text{Speed}(s) = \frac{Labour(\partial)}{Time(t)}$$

$$> \quad Time(t) = \frac{Labour(\partial)}{Speed(s)} \quad - ❶$$

But labor α Difficulty (b) -❷

$$\alpha \frac{1}{Ability(\alpha)} \quad - ❸$$

Using a constant value k we can get

Labor=b/α x k - ❹

By considering log values of ❹

$\beta = \ln b - \ln a + \ln k - \ln s$ - ❺

By substituting❺ to log normal form we can get

P=$\frac{a}{t\sqrt{2\pi}}$ exp[-$\frac{1}{2}$a(ln t-(ln b – ln a- ln s))] ❻

Developing a new unsupervised clustering algorithm

The prime objective of clustering is to segregate groups with similar traits. Usually, clustering algorithms deal with a single process. However, in Teaching and Learning Process, there are two separate processes which have to be considered.

1. The learning process basically depended on student's ability. In addition, the response time (RT) and guessing factor are also affected.
2. Usually, the teacher started to teach any lesson from the basic level. Therefore, the difficulty level of the lesson is started from the bottom and extended to the high level of difficulty.

But in study about both supervised and unsupervised algorithms, there should be a mechanism to find the next level of the cluster point. Thus, for the new algorithm, there should be a way of advancing to the next clustering level. As it is a psychometric measurement, Greatest Common Divisor (GCD) can be utilized as a factor of the increment. The new algorithm does not need a specific number of clusters to be given, before performing the clustering process and it is able to find the optimal number of clusters during the clustering process. For this task, the natural phenomenon of understanding of human mind is used.

Input: k: number of clusters (for dynamic clustering initialize k=2)

Fixed number of clusters = yes or no (Boolean).

Output: A set of clusters.

Method

Teacher Phase

1. Suppose that, the difficulty of the lesson presented by teacher T1 –>b1
2. At the initial level at the mean ability of the class a1

Then, X1= a1

3. After presenting the lesson with difficulty b1 by the teacher T1, the mean ability of the class has been changed into anew then;

The difference of the mean D= (anew-a1)

4. Suppose that student Xold,i status is transformed to Xnew,i for i=1 to n
5. Therefore, the function of the transformation

Xnew,I = Xold,I + D

Student Phase

1. Suppose that, C1 & C2 are cluster centers based in probability ranges. Xnew,I & Xnew,j are two students in the class.

Then,

2.From P1 to Pn do , (n ∈ N)

3.P1= Pmin

4.If P(c1) > Xnew,I and if P(c1) < Xnew,j then

5.Xnew,j ∈ {C1}

6.Else Xnew,I ∈ {C2}

7.Until Pn =Pmax

Loop

Results and Discussion

Application of Two Parameter Item Response Theory (IRT) Model:

Case Study: MCQ paper with 20 questions (459 students)

First the difficulty level of each question is calculated using Rasch Model. Figure 1 shows the initial difficulty level of each question.

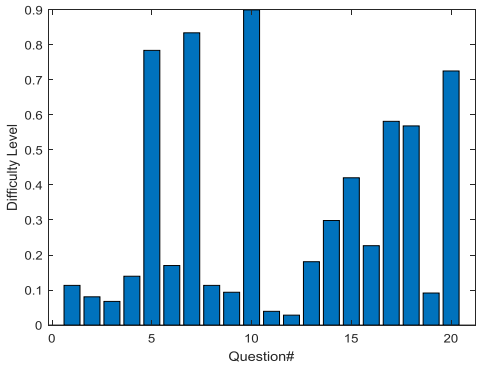

Figure 2. Initial Difficulty Level of Each Question

From figure 2, it can be seen that question numbers 5,7,10 and 20 are considerably difficult than the other questions. Further, it can be seen that the question numbers 1, 2, 3, 4, 6, 8, 9, 11, 12 and 19 are relatively easier than the other question. However, the difficulty level of a particular question is based not only on difficulty level, but also on the discrimination parameter. The difficulty level is adjusted using the MLE algorithm. After adjustment, each student was represented by a twenty dimensional feature vector consisting of the weighted marks for each question. Then, this 20 dimensional feature space was mapped to a 2 dimensional feature space using Principle Component Analysis (PCA) for visualization purposes.

Application of New Time based IRT Model with proposed Time for question parameter:
Case Study: MCQ paper with six questions (46 students)
To test the newly proposed time based Item Response Theory equation which is mentioned in Equation 06. Earlier used data set was used with time data. Calculation process was performed as earlier with new equation, and the results were shown in Figure 3, in 3D space.

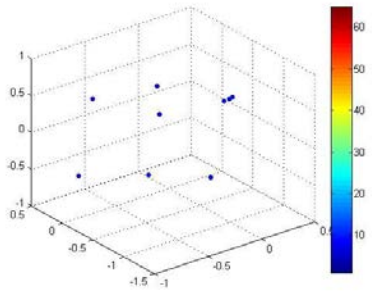

Figure 3. Representation of Psychometric values in new IRT equation in 3D Space

Testing the new two phase clustering mechanism.
Case Study: MCQ paper with six questions (46 students)
To test the working level of the new two phase clustering algorithm, the same data set has been utilized for the clustering. By applying the algorithm, there are 14 clusters as shown in figures 4 and 5.

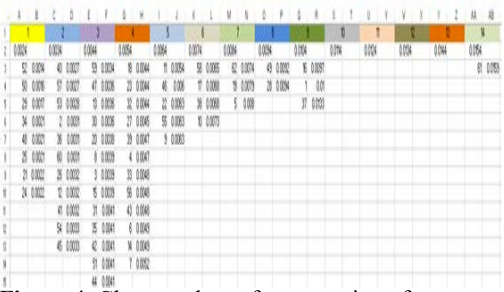

Figure 4. Cluster values after execution of new clustering algorithm

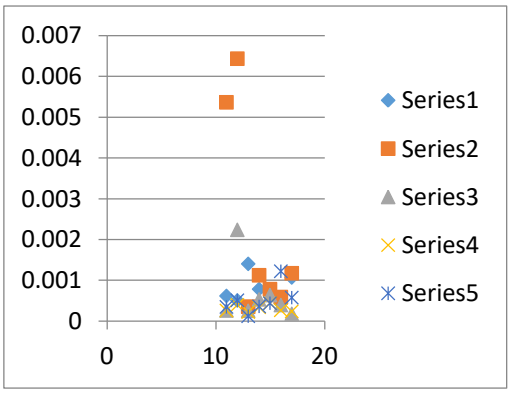

Figure 5. Cluster values after execution of new clustering algorithm – Graphical View

Conclusion

The research presented in this thesis makes contributions which are empirical, theoretical and methodological in Computer Science and Education Science.

The main empirical contribution is that this thesis provides an analysis to find out the root causes for lower results in G.C.E (Ordinary Level) Mathematics. However, none of the previous studies have examined Primary Mathematics Knowledge as a root cause for the poor results in G.C.E (Ordinary Level) Mathematics. In addition to that, this thesis extends the current literature on the importance of E Learning in Mathematics education. E learning is a remedy for lack of Primary Mathematics teachers in the North Central Province. Also, two research papers were published in international journals in order to contribute the literature. The findings of this research provide and contribute several theoretical perspectives. The first one is, the adding new parameter to the Item Response Theory. The Second thing is, a new web based framework was developed for the Agent based E learning System. This new web frame work has been designed especially for the new Agent based E learning System. The main methodological Contribution of this study is the development of a new two phase unsupervised clustering algorithm. This new unsupervised clustering method has been designed for the Agent based E learning System to make the system automatic. This makes the system cluster students according to their psychometric measurements. Before developing this new algorithm, Particle Swarm Optimization and was tested in order to check the compatibility to use in the Agent based E learning System. But it was realized that this clustering method is not suitable for two phase simultaneous process like teaching and learning. Therefore the new clustering method was developed and tested using the real data set.

Selected References

Department of Examination. (2011). *Statistical handbook, 2008 – 2010*. Department of Examination, Colombo. Department of Examination.

Chong, H. Y. *A Simple Guide to the Item Response Theory (IRT) and Rasch Modeling*: Retrieved from http://www.creative-wisdom.com.

Item Response Theory. Retrieved from http://echo.edres.org:8080/irt/.

Rasch, G. (1960/1980). *Probabilistic models for some intelligence and attainment tests*. Copenhagen, Danish Institute for Educational Research, Expanded edition (1980) with foreword and afterword by B.D. Wright. Chicago. The University of Chicago Press.

IRT Tutorials. (2008). Retrieved January 4, 2018, fromhttps://web.archive.org/web/20041210140342/http://work.psych.uiuc.edu/irt/tutorial.asp.

Ching-Yi. C. and Fun. Y. (2004). Particle swarm optimization algorithm and its application to clustering analysis. *IEEE International Conference on Networking, Sensing and Control*, 2, 789-794.

Das, S., Abraham, A, and Konar, A. (2012). Automatic clustering using an improved differential evolution algorithm. *IEEE Transactions on Systems, Man and Cybernetics, Part A: Systems and Humans*, 38, 218-237

Selim, S. Z., and Alsultan, K. (1991). A simulated annealing algorithm for the clustering problem. *Pattern Recognition*, 24(10), 1003-1008.

Selim, S. Z., & Ismail, M. A. (1984). K-means-type algorithms: A generalized convergence theorem and characterization of local optimality. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 1, 81-87.

Publications

Lakshman, B., Wijekulasooriya, J. and Sandirigama, M. (2018). Implementing new Two Phase Unsupervised clustering algorithm in E Learning. *International Journal of Science and Innovative Engineering & Technology (IJSIET) Journal*.

Lakshman, B., Wijekulasooriya, J. and Sandirigama, M. (2016). Applying K means Clustering Algorithm in Item Response Theory. *International Journal of Advanced Research in Computer Science and Software Engineering*, 6(2).

Thesis Reference

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APPEARANCE BASED ONLINE VISUAL OBJECT TRACKING

T. Kokul *graduated in 2009 with a B.Sc. special in Computer Science from the University of Jaffna. He has started his M.Phil. at the PGIS in 2014 and then got a joint-Ph.D. scholarship under the Sri Lankan-Queensland University of Technology joint Ph.D. programme. He has completed his PhD at Queensland University of Technology, Australia in 2019. At present, he is serving as a Senior Lecturer in the Department of Physical Science, Faculty of Applied Science, Vavuniya Campus of the University of Jaffna.*

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Large amounts of videos are captured every second all around the world and there is an urgent need in many applications to develop computer vision based systems that process such videos automatically in real-time. Appearance based visual object tracking is the task of automatically estimating the state (location, size and orientation) of an unknown target in a video sequence by using the appearance cues of the target while only the initial state is given. Although it has been investigated for several years, reaching the tracking accuracy of humans with real-time speed still remains a challenging problem since there exist enormous variations, which strongly influence both the target and background.

This study investigates appearance based online visual object tracking by using hand-crafted and deep features. Hand-crafted feature based trackers capture the target appearance by using a predefined algorithm based on expert knowledge. In contrast, deep feature based trackers learn the target appearance from raw
images. Most of the hand-crafted trackers are struggling to achieve state-of-the art accuracy. On the other hand, deep trackers are facing difficulties to achieve real-time tracking speed since they employ an extensive set of parameters. Over- fitting is also another major issue in current deep trackers. This research addresses these challenges in appearance based online object tracking.

The major objective of this study is the development of a high performance tracker with real-time tracking speed to satisfy the demands of real-world applications. To achieve this, the scope was constrained to the following sub objectives:

(I) To evaluate the traditional visual tracking approaches to find the research gap and develop a robust hand-crafted feature based tracking approach at the initial stage of this study

(II) To further improve the tracking accuracy after the emergence of deep learning, investigating the internal properties of Convolutional Neural Networks (CNN) for a deep classification based tracking framework

(III) To research and develop a novel deep similarity learning and classification based tracking framework to successfully balance the tracking accuracy and speed

(IV) To research and develop a novel deep similarity learning based real-time tracking framework for high-accuracy and high speed tracking.

Methodology

In this study, a number of original contributions are made in the field of appearance based visual object tracking by using hand-crafted and deep features. Several novel tracking frameworks have been proposed, which can provide value to both scientific and industrial communities.

A Traditional Tracking Framework using Aggregate Channel Features (ACF) and Particle Filter

A novel real-time, hand-crafted feature based multi-object tracking framework is proposed at the beginning of this study. The proposed tracker combines the cues from a pre-trained object detector, an online trained target-specific detector, and a particle filter framework to predict the location of a target. Coincident detection between these three models are used to increase the accuracy and to reduce the false tracking. The Aggregate Channel Feature (ACF) is used to capture the target appearance and an AdaBoost classifier is used to locate the targets by using a tracking-by-detection technique.

A Deep Tracking Framework using Gate Connected Convolutional Neural Network

In the next stage of this study, novel convolutional neural network architecture is proposed for model-free object tracking. While the learning capability of a CNN increases with its depth, their spatial information is diluted in deeper layers which hinders its important ability to localize the targets. The proposed CNN architecture connects the early and deeper convolutional features with a gate layer and hence it manages the trade-off between learning capability and spatial information loss in an end-to-end manner. In this framework, a novel domain adaptation technique is utilised to train the CNN architecture online with fewer number of samples. The proposed technique is used to finetune the CNN architecture with fewer number of samples and prevents the effect of over-fitting.

A Deep Classification Tracking Framework by using Similarity Patch Matching Techniques

In this framework, a novel convolutional neural network architecture is proposed to successfully manage the trade-off between accuracy and speed of deep trackers. Consecutive similar frames are processed with a similarity matching technique, and dissimilar frames are processed with a classification approach within the CNN architecture. This approach increases the tracking speed by avoiding unnecessary model updates through the measurement of similarity between adjacent frames, while the accuracy is maintained by adapting a classification approach when needed, with deeper level features.

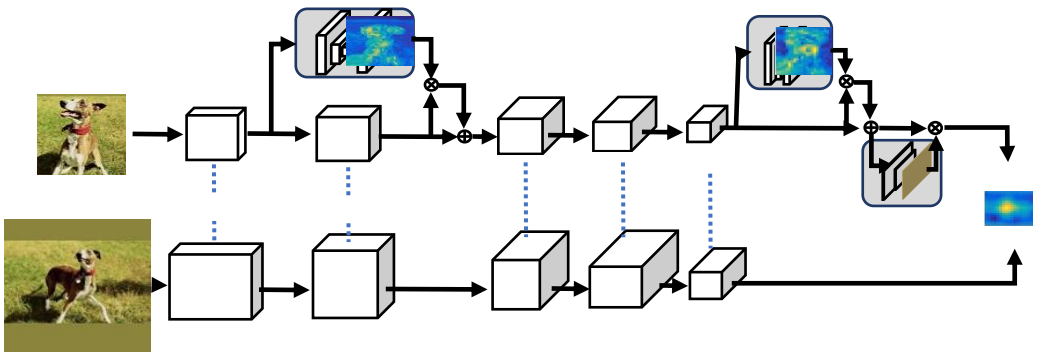

Figure 1. Deep similarity tracking framework by using Target-Specific Siamese Attention Network. It has two residual attention modules to feed target-specific information to similarity tracking at low-level and high-level feature representations. In addition, it has a channel attention module to boost target-specific informative channels, which is stacked at the end of the exemplar branch

A Deep Similarity Tracking Framework by using Target-Specific Siamese Attention Network
A robust object tracker ideally should have state-of-the-art accuracy while maintaining real-time tracking speed. The accuracy of similarity tracking approaches can be improved by feeding the target-specific information online. To achieve this objective, we propose a target-specific Siamese attention network for object tracking. As shown in figure1, the proposed approach uses different kinds of attention modules to capture the different context of target-specific information online. Then the captured knowledge is used to feed the target-specific cues in template matching and hence the discriminative power of similarity tracking is improved. In addition, the proposed approach reduces the impact of noise in the target template and hence the performance of similarity searching is improved. The proposed residual and channel attention modules capture the different context of target information and then feed the learned knowledge at different levels of representation of similarity tracking to improve the accuracy. These attention modules can be stacked with existing deep similarity trackers without interrupting their capability. Further, an online data utilisation mechanism is proposed to capture all the informative target-specific cues in similarity tracking. A hard-negative mining based training is used to identify the distracting objects/regions and to remove their impact in template matching.

Results and Discussion
A Traditional Tracking Framework using Aggregate Channel Features (ACF) and Particle Filter
This framework has been trained in Caltech Pedestrian dataset and showed performance against state-of-the-art trackers while maintaining the tracking speed in real-time.

A Deep Tracking Framework using Gate Connected Convolutional Neural Network
This framework has been trained on VOT benchmark sequences and evaluated on OTB2013 benchmark. It showed state-of-the-art accuracy.

A Deep classification Tracking Framework by using Similarity Patch Matching Techniques This framework showed state-of-the-art accuracy and near real-time tracking speed on OTB2013 benchmark.
A Deep Similarity tracking framework by using Target-Specific Siamese Attention Network
This framework trained on ImageNet video detetion dataset and evaluated on OTB, and VOT benchmarks. It showed state-of-the-art accuracy with real-time tracking speed.

Conclusion
This study proposed several frameworks to address the challenges and limitations in appearance based single object tracking. Traditional machine learning and deep learning based algorithms have been proposed and evaluated on publicly available benchmark datasets. Experimental results showed that proposed frameworks showed state-of-the-art performances.

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Selected References
Wu, Y., Lim, J. and Yang, M. H. (2015). Object tracking benchmark, *IEEE Trans. Pattern Anal. Mach. Intell*., 37 (9), 1834–1848.

Li, X., Hu, W., Shen, C., Zhang, Z., Dick, A. and Hengel, A. V. D. (2013). A survey of appearance models in visual object tracking. *ACM transactions on Intelligent Systems and Technology (TIST)*, 4 (4), 58.

Bertinetto, L., Valmadre, J., Henriques, J., Vedaldi, A. and Torr, P. (2016). Fully convolutional siamese networks for object tracking. In *European Conference on Computer Vision*., 850–865.

Nam, H. and Han, B. (2016). Learning multi-domain convolutional neural networks for visual tracking. In *Proc. CVPR*, 4293–4302.

He, A., Luo, C., Tian, X. and Zeng, W. (2018). A twofold Siamese network for real-time object tracking, In *Proc. IEEE Conf. Comput. Vis. Pattern Recognit*. 4834–4843.

Publications
Kokul, T., Fookes, C., Sridharan, S., Ramanan, A. and Pinidiyaarachchi, U.A.J. (2019). Target-specific siamese attention network for real-time object tracking. In *IEEE Transactions on*

Information Forensics and Security, 15, 1276 – 1289.

Kokul, T., Fookes, C., Sridharan, S., Ramanan, A. and Pinidiyaarachchi, U.A.J. (2018). Deep match tracker: classifying when dissimilar, similarity matching when not. *IEEE International Conference on Image Processing (ICIP), Athens, Greece*, 2730-2734.

Kokul, T., Fookes, C., Sridharan, S., Ramanan, A. and Pinidiyaarachchi, U.A.J. (2017). Gate connected convolutional neural network for object tracking. In *IEEE International Conference on Image Processing (ICIP)*, Beijing, China. 2602-2606.

Kokul, T., Ramanan, A. and Pinidiyaarachchi, U.A.J. (2015). Online multi-person tracking-by-detection method using ACF and particle filter. *Seventh IEEE International Conference on Intelligent Computing and Information Systems*, Cairo, Egypt, 529-536.

Thesis Reference

Kokul, T. (2019). Appearance Based Online Visual Object Tracking. Ph.D. Thesis, Queensland University of Technology, Australia.

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Vision of the PGIS

To be an internationally renowned Centre of Excellence in Asia for Postgraduate Education and Research in Science

Mission of the PGIS

The mission is to the development of the knowledge-based society with social sensitivity, ethical rectitude and economic prosperity through postgraduate level education and research. Also, to disseminate knowledge in an intellectually stimulating, efficiently, managed, harmonious academic environment

Scope of "PGIS Postgraduate Research Highlights"

The Postgraduate Institute of Science (PGIS) of University of Peradeniya was established in 1996. With the establishment of the PGIS, postgraduate research in science-based fields received a new lease of life. During the last 23 years, PGIS has grown into a strong robust tree with spreading branches representing the major disciplines in Science. It harbours a scientific community from across faculties, embracing scientists in many specialties. With the 7th Annual Research Congress of the PGIS, being held this year, the researches of postgraduates who received their M.Phil. and Ph.D. degrees in 2019, have been brought to life in the form of Postgraduate Research Highlights. The recipients of the postgraduate research degrees are also brought to life in a short personal profile and a photograph. Thus, the unseen young researchers behind the study make an appearance in this publication. The PGIS of the University of Peradeniya is privileged to be a national institute giving leadership and guidance not only to the young and upcoming researchers of the University of Peradeniya but also to researchers in other universities, particularly those in newly established peripheral universities. In an era where research standards are declining, scarifying quality for quantity, it is timely that postgraduate research output is brought to light for its improvement, applications and future collaborations. The publication, PGIS Postgraduate Research Highlights has been launched to meet these goals.