

CHEMISTRY

I N S R I L A N K A



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32nd batch of 106 Graduate Chemists at the Eleventh Convocation held
at Eagles' Lakeside Convention Centre on 19th February 2015



40th Batch of 80 DLIC Diplomates at the Eleventh Convocation



Chemistry in Sri Lanka

ISSN 1012 - 8999

The Tri-Annual Publication of the Institute of Chemistry Ceylon

Founded in 1971, Incorporated by Act of Parliament No. 15 of 1972

Successor to the Chemical Society of Ceylon, founded on 25th January 1941

Vol. 32 No. 2

May 2015

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Theme for the year -

"Chemical Sciences in Food Safety and Security"

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Outline of our Institute

The Institute of Chemistry Ceylon is a professional body and a learned society founded in 1971 and incorporated by act of Parliament No. 15 of 1972. It is the successor to the Chemical Society of Ceylon which was founded in 1941. Over 50 years of existence in Sri Lanka makes it the oldest scientific body in the country.

The Institute has been established for the general advancement of the science and practice of Chemistry and for the enhancement of the status of the profession of Chemistry in Sri Lanka. The Institute represents all branches of the profession and its membership is accepted by the government of Sri Lanka (by establishment circular 234 of 9-3-77) for purposes of recruitment and promotion of chemists.

Corporate Membership

Full membership is referred to as corporate membership and consists of two grades: **Fellow (F.I.Chem.C.)** and **Member (M.I.Chem.C.)**

Application for non-corporate membership is entertained for four grades: **Associate (former Graduate) (A.I.Chem.C.)**, **Licentiate (L.I.Chem.C.)**, **Technician (Tech.I.Chem.C.)** and **Affiliate Member**.

Revision of Membership Regulation

All Special Degree Chemists can now apply directly to obtain **Associate (Graduate) Membership**. Three year B. Sc. Graduates (with an acceptable standard of Chemistry) can

- directly become **Licentiate**
- obtain corporate membership in a lesser number of years.

Tech.I.Chem.C.

Those who have passed the DLTC examination or LTCC examination or have obtained equivalent qualification and are engaged in the practice of Chemistry (or chemical sciences) acceptable to the Council are entitled to the designation **Tech.I.Chem.C.**

Members/Fellows are entitled to the designation of **Chartered Chemist (C.Chem.)** on establishment of a high level of competence and professionalism in the practice of chemistry and showing their commitment to maintain their expertise.

All corporate members (Members / Fellows) are entitled to vote and become Council/ Committee members whether Chartered Chemists or not.

Membership Applications

Any application for admission to the appropriate class of membership or for transfer should be made on the prescribed form available from the Institute Office.

Current Subscription Rates

Fees should be paid on 1st of July every year and will be in respect of the year commencing from 1st July to 30th June

Fellow	Rs. 1500
Member	Rs. 1500
Associate	Rs. 1200
Licentiate	Rs. 1000
Technician	Rs. 500
Affiliate	Rs. 1000
Membership for Life	Rs. 15000

Entrance Fee

All the grades	Rs. 1000
Processing Fees*	Rs. 500
Processing Fee for Chartered Chemist designation	Rs. 1000
Institutional Members	Rs. 2500

*per application for admission/transfer to any grade

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CHEMISTRY IN SRI LANKA

Chemistry in Sri Lanka is a tri-annual publication of the Institute of Chemistry Ceylon and is published in January, May and September of each year. It is circulated among the members of the Institute of Chemistry and students of the Graduateship/DLTC course and libraries. The publication has a wide circulation and more than 1500 copies are published. Award winning lectures, abstracts of communications to be presented at the annual sessions, review papers, activities of the institute, membership news are some of the items included in the magazine.

The editor invites from the membership the following items for publication in the next issue of the Chemistry in Sri Lanka which is due to be released in September 2015.

- Personal news of the members
- Brief articles of topical interests
- Forthcoming conferences, seminars and workshops
- Latest text books and monographs of interest to chemists

All publications will be subjected to approval of the 'Editorial and Publicity Committee' and the Council of the Institute of Chemistry Ceylon.

Further, prospective career opportunities for chemists, could be advertised in Chemistry in Sri Lanka at a nominal payment. The editor welcomes from the members suggestions for improvement of the publication.

Providing safe and wholesome food for the nation – the role of the Chemist

Professor Siromi Samarasinghe

Department of Chemistry, University of Sri Jaywardenepura.



In keeping with the current year's theme, “Chemical sciences in Food Safety and Security”, the active participation of chemists in ensuring safe and nutritious food for the consumer is a pressing need to a developing country such as Sri Lanka.

The food chain, or the food system extends from the producer to the consumer, and is often referred to as “Farm to Fork”. Within the food chain, food security can be addressed at three levels: ensuring adequate availability, ensuring adequate nutrition and assuring the safety of the food supply.

The best and the most effective method of assuring food safety is to establish a systematic approach to raw material screening and to the control of food manufacturing practices and handling procedures to ensure lowest possible risks to the consumer.

Prevention of food borne diseases and other hazards associated with the food supply is of serious concern and requires the combined efforts of all sectors involved in the supply and manufacturing of food and food products.

Good agricultural practices (GAP) will ensure safe and wholesome food to the consumer. For on-farm processes, clean water and products free from contaminants are essential.

The Food industries have improved over the years. Good Manufacturing Practices (GMP), standards such as Global Food Safety Initiative (GFSI), International Food Standard (IFS), British Retail Consortium (BRC), Safe Quality Food (SQF) 2000 and International Organization for Standardization - ISO 22000:2005 among others are now followed by most Food Industries.

Food can never be proven to be entirely safe or entirely harmful. Food Safety is defined as “The practical certainty that injury or illness will not result from the consumption of food, either short term or long term”. Within this definition one can only say that food may not be safe for consumption due to the presence of potentially harmful substances in them.

Potentially harmful substances are the potential food hazards, which are any biological, chemical or physical agent in food, with the potential to cause an adverse health effect. These can enter the Food Chain at

any stage. At the growing stage: fruits, vegetables and other plant products are subjected to insect infestations, contamination from pesticides and agrochemicals, exposure to harmful substances used for ripening of fruits and fumigants if good agricultural practices are not followed. Many local farmers are either ignorant of these requirements or do not follow GAP due to lack of knowledge or awareness, or perhaps due to financial constraints.

Sri Lanka being a tropical country with high humidity provides the ideal environment for the growth and activity of harmful microorganisms that cause food poisoning and food borne illnesses. Safety procedures have to be strictly followed by the food industries to eliminate risks of contamination of raw material during handling, manufacturing and storage in order to protect the consumer from such hazards.

Hazard Control Critical Points (HACCP) is an essential food safety procedure that should be followed by all food processors and manufacturers, to ensure safe food for the consumer.

In the recent past there have been many instances of food contamination and adulteration in Sri Lanka. The addition of melamine to milk powder, the presence of DCD in milk are two examples. Many street vendors use harmful food dyes which are cheaper than food grade colouring. Formalin is used by fish vendors to extend the shelf life of fish, while the fish itself may be contaminated with mercury compounds. Such adulterants and contaminants could be easily detected using modern analytical techniques and instrumentation available to the chemist.

Harmful chemicals are produced from frying oils, when used for repeated frying, and also during grilling and barbecuing. Polycyclic aromatic hydrocarbons formed are a health hazard which the consumer may not be aware of. Harmful heterocyclic amines are produced when meat and fish are subject to very high temperatures. Also there are natural toxicants in food such as mushrooms, shellfish and certain vegetables.

Consumers have to be aware of food allergies from foods such as peanuts, prawns, strawberries, pineapple and also the reactions caused by certain additives like sulphur dioxide and sulphites added to soft drinks and fruit products. The latter substances are potentially harmful to individuals who suffer from asthma. School children, housewives and the general public need to be educated in these aspects of food safety through

workshops, popular lectures and training programmes in which chemists should participate.

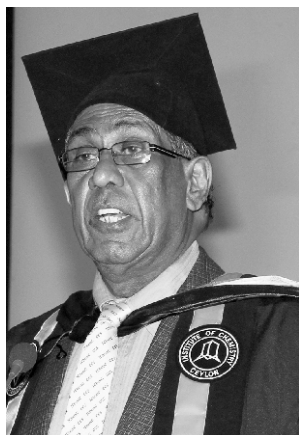
Chronic Kidney Disease of unknown etiology (CKDu) prevalent in the North Central Province affecting mostly the farming community, has been a topic of interest in Sri Lanka. The chemists will be able to provide the answers to the many questions that have come up in relation to the causative factors of CKDu - the hardness of the water, presence of heavy metals, nephrotoxins entering the food chain, environmental factors - which require further studies to ascertain if a combination of factors are responsible.

Iron deficient anaemia, vitamin A deficiency and iodine deficiency are three key micronutrient deficiencies in Sri Lanka which are of public health significance. Current iodine deficiency disorder (IDD)

rates are lower in Sri Lanka compared with other countries in the region. The fortification of salt with iodine has helped towards this achievement. Dental fluorosis has been identified as an endemic problem in the dry zone of Sri Lanka. Chemists could help improve the current methods used for the defluoridation of water in these areas.

Food scientists, nutritionists and technologists have a vital role to perform in ensuring safe and wholesome food to the nation. The chemists could contribute their knowledge and expertise at every stage of the food supply chain, work together with the agricultural sector, food manufacturing industries, marketing sector, hotels and catering establishments to achieve the goal of providing safe and wholesome food for the nation.

In memory of Professor J N O Fernando



Professor J N O Fernando passed away on 2nd March 2015 after a brief illness, while he was serving as the Honorary Rector of the College of Chemical Sciences, Institute of Chemistry Ceylon. Professor Fernando was the founder coordinator of the Graduateship programme in Chemistry which commenced in 1979. He took a keen interest and devoted his time and energy to develop both the Graduateship in Chemistry (GIC) and the Diploma in Laboratory Technology (DLTC) programmes to be the high quality and well-recognised programmes they are today. He also took the initiative to provide infrastructure facilities including the library, laboratories and a new building to conduct courses successfully. He also served as the Honorary Dean and the Chairman of the Academic Board of the College of Chemical Sciences. Professor Fernando joined the Institute in 1973 and was an active member of the Council till his sudden demise. He was the General Secretary from 1978 to 1981 and the President of the Institute of Chemistry Ceylon from 1984 for two consecutive years. He was the President of the Sri Lanka Association for the Advancement of Science (2001) and a Fellow of the National Academy of the Sciences of Sri Lanka.

Professor Fernando's sudden demise is an immense loss to the Institute, the College and the science community in Sri Lanka. The Council of the Institute has decided to publish a special issue of Chemistry in Sri Lanka dedicated to Professor Fernando.

May Professor Fernando's soul rest in peace !

Cover Page

The cover page photograph shows the Graduate Chemists after the 11th Convocation of the College of Chemical Sciences, Institute of Chemistry Ceylon, held at Eagles Lakeside Banquet & Conventional Centre on 19th February 2015. This was the 32nd batch and 106 students were formally awarded Graduate Chemist status and thereby increasing the overall production to a total of 1075. More formal photographs of the Convocation are on inner & outer cover pages.

Institute of Chemistry Ceylon

Forty Fourth Annual Sessions and Seventy Fourth Anniversary Celebrations 2015

Inauguration of the 44th Annual Sessions, Institute of Chemistry Ceylon

Wednesday, June 17th 2015
Centre for Banking Studies, Rajagiriya

8.00-8.30 am	Arrival of Members and Guests (<i>Refreshments will be served</i>)
8.30 am	Ceremonial Procession of the Council Members and Past Presidents
8.40 am	Inauguration by lighting of the Oil Lamp and playing the National Anthem
8.45 am	Welcome Address by Prof H D Gunawardhana <i>President, Institute of Chemistry Ceylon</i>
8.50 am	Presidential Address
9.10 am	Address by the Guest of Honor Prof. Upali Samarajeewa <i>Past President, Institute of Chemistry Ceylon</i>
9.30 am	Address by the Chief Guest Mr. Athauda Jayawardena <i>President, Organisation of Professional Associations of Sri Lanka</i>
9.50 am	Presentation of Awards, Prizes and Certificates <i>Dr. C L de Silva Gold Medal Award</i> <i>Chandrasena Memorial Award</i> <i>Kandiah Memorial Graduateship Award</i> <i>Professor M U S Sultanbawa Award for Research in Chemistry 2014 - Mr. S C D Fernando</i> <i>Special Service Awards - Mr. N I N S Nadarasa, Ms. A C Wijesuriya & Mr. U J N Chandana</i> <i>Graduateship Examination in Chemistry - Scholarships Prizes and Awards</i> <i>All Island Interschool Chemistry Quiz Prizes</i>
10.40 am	Dr. C L de Silva Gold Medal Award Lecture
11.00 am	Chandrasena Memorial Award Lecture
11.20 am	Vote of Thanks Mr. K R Dayananda <i>President Elect, Institute of Chemistry Ceylon</i>
11.30 am	Close of Ceremony
12.00 noon	Annual General Meeting at PPGL Siriwardene Auditorium, Adamantane House (<i>for Corporate Members only</i>)

Chief Guest at the 44th Annual Sessions

Mr. Athauda Jayawardena



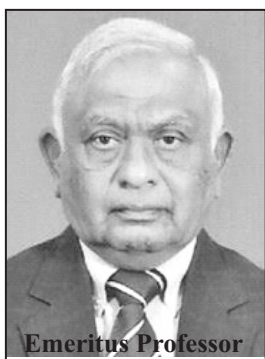
Mr. Athauda Jayawardena

Mr. Athauda Jayawardena is the current President of the Organisation of Professional Associations of Sri Lanka (OPA). He obtained a B.Sc. (Agriculture) degree from the Faculty of Agriculture, University of Peradeniya in 1976. He has worked as a Research Assistant attached to the Department of Agric Economics and Extension of the Faculty of Agriculture, Peradeniya. In this capacity he has worked under a project on "Constraints to High Rice Yields in South East Asia" sponsored by the International Rice Research Institute (IRRI). Then in 1979, he has joined Chemical Industries (Colombo) Ltd (CIC) as a Product Executive. He was promoted on to the Main Board of Directors of CIC in 1995. He has resigned from the Board of Directors of CIC and formed his own company, Innovative Pesticides marketing (Private) Limited, in 2002. He is the Director, CEO of this company.

He has served as the Chairman of the Foreign Employment Agency (Pvt.) Ltd in 2005 and as the Working Director of the Foreign Employment Bureau in 2006. He has also served as a member of the Councils of the University of Peradeniya and Wayamba University of Sri Lanka, and the Standing Committee on Agriculture, Veterinary Medicine and Animal Sciences of the University Grants Commission. At present, he serves as a member of the Council of the Open University of Sri Lanka and Board of Study in Plant Protection of the Postgraduate Institute of Agriculture.

Guest of Honour at the 44th Annual Sessions

Emeritus Professor Upali Samarajeewa



**Emeritus Professor
Upali Samarajeewa**

Emeritus Professor Upali Samarajeewa holds a Bachelors' degree in Chemistry, and PhD in microbiology from University of Peradeniya. He served 5 years as a research officer at Coconut Research Institute and 30 years as a teacher at University of Peradeniya. He worked as a UNDP Fellow at the Tropical Products Institute, London and a Senior Fulbright-Hays Senior Research Scholar and Adjunct professor at the University of Florida, USA.

Professor Samarajeewa was the recipient of Institute Chemistry Gold medal for his research on "coconut products", General Research Committee Award of the SLAAS for Most Outstanding Research Contribution to Sri Lankan Science for research on "aflatoxins", National Award for Agriculture Research from Council for Agricultural Research Policy and Ministry of Agriculture for "Investigations on deposition, formation and control of polycyclic aromatic hydrocarbons in coconut kernel products during processing in relation to food safety", and two merit awards for research from National Science Foundation. He has more than 200 research publications to his credit.

He was the President of the Institute of Chemistry Ceylon in 1999, and President of the Sri Lanka Association for the Advancement of science in 2000. Professor Samarajeewa was the founder head of the Department of Food Science & Technology at the University of Peradeniya, and founder Public Analyst in the City Analyst laboratory in Kandy.

He has served in 23 countries as an International Consultant for United Nations Industrial Development Organization, World Bank, and Asian Development Bank in the fields of chemical and microbiological laboratory accreditation, food safety, and curriculum development in Food Science and Technology.

Professor Samarajeewa is the President of the Institute of Food Science & Technology Sri Lanka currently.

Theme Seminar on
“The Role of Chemistry in Food Safety and Food Security”
18th June 2015

Venue: PPGL Siriwardene Auditorium, Adamantane House, Rajagiriya

Programme

8.30 -9.00 am	Registration
9.00 am	Inauguration and playing the National Anthem
9.05 am	Welcome Address by Prof H D Gunawardhanana
9.15 am	Address by the Chief Guest Food Safety Mr. E G Somapala <i>Former Government Analyst</i>
10.00 am	Self Sufficiency & Resource Use Dr. W M W Weerakoon <i>Director, Field Crop Research & Development Institute, Department of Agriculture</i>
10.40 am	TEA
11.00 am	Toxic Chemicals in Food Dr. Sirimal Premakumara <i>Former Director, Industrial Technology Institute</i>
11.40 am	Role of Chemist in Ensuring Food Safety and Security Professor Upali Samarajeewa <i>Past President, Institute of Chemistry Ceylon</i>
12.20 am	LUNCH
1.30 pm	Post-harvest Technology with Special Emphasis on Transportation Prof T R Ariyaratne <i>Emeritus Professor, Department of Physics, University of Colombo</i>
2.10 pm	Food Production and Food Security Dr. Noble Jayasuriya <i>Programme Director, The Coordinating Secretariat for Science, Technology and Innovation (COSTI)</i>
2.50 pm	TEA
3.20 pm	Food Safety and Food Security Dr. Sachie Pinnawala <i>Scientist, The Coordinating Secretariat for Science, Technology and Innovation (COSTI)</i>
4.00 pm	Food Security and Water Quality Prof H D Gunawardhana <i>Emeritus Professor of Chemistry, University of Colombo and President, Institute of Chemistry Ceylon</i>
4.40 pm	Vote of Thanks

Technical Sessions

Venue: P P G L Siriwardene Auditorium, Adamantane House, Rajagiriya

Time : 2.00 p.m. – 4.45 p.m.

Date: 17th June 2015

Time	Title	Authors
2.00 - 2.30 pm	Kandiah Memorial Graduateship Award	
2.30 - 2.45 pm	Selenium Content in Daily Meals Consumed by Sri Lankans -Apreliminary study and H P Weeraratna	K M S D Kiridena, D S M De Silva, Sukumal Wimalasena, A T Kannangara
2.45 - 3.00 pm	<i>In vitro</i> 5- Lipoxygenase enzyme inhibitory and anti-oxidant activities of Sri Lankan medicinal plant leaves: <i>Bacopa monieri</i> , <i>Melaleuca</i> and <i>Sphaeranthus indicus</i>	R Samarasekara and H D S M Perera
3.00 - 3.30 pm	TEA BREAK	
3.30 - 3.45 pm	Isolation and Molecular Characterization of Sri Lankan <i>Bacillus Thuringiensis</i> for potential Lepidopteran Activity	R Y Baragamaarachchi, O V D S J Weerasena and R Samarasekara
3.45 - 4.00 pm	Elastase, tyrosinase inhibitory and antioxidant activity of <i>Rubia cordifolia</i>	G D Liyanarachchi and R Samarasekara
4.00 - 4.15 pm	Biochemical and molecular characterization of probiotics from fermented traditional rice varieties	J T Kotelawala, R Samarasekara, O V D S J Weerasena and D M W D Divisekara
4.15 - 4.30 pm	Chemical and microbiological analysis of toothpaste available in leading supermarkets in Sri Lanka	K G Sapumohotti, S D M Chinthaka, J G P S Ubesena, S P Deraniyagala and Manel Perera
4.30 - 4.45 pm	Synthesis of some Cu(I) complexes with bidentate N And P Donors	Sarath D Perera

Venue: P P G L Siriwardene Auditorium, Adamantane House, Rajagiriya

Time : 8.30 a.m. – 11.45 a.m.

Date: 19th June

Time	Title	Authors
8.30 - 8.45 am	Synthesis and biological studies of <i>Fac</i> - [ReL(CO) ₃] ₂ BF ₄ ; L=N(SO ₂ piperidinyl) dipicolylamine	S A A S Subasinghe, I C Perera and T Perera
8.45 - 9.00 am	Nitric oxide scavenging activity of the herbal formulation Nawarathne Kalka used in traditional medicinal systems in Sri Lanka for the treatment of rheumatoid arthritis	M G D T Karunarathne, P K Perera, C Udawatte and S C D Fernando
9.00 - 9.15 am	Antioxidant and Cytotoxic Activities of Proanthocyanidins of the Bark <i>Thespesia populnea</i> (L.)	Chayanika padumadasa, Ajita M Abeysekara, Ira Thabrew and Gayathri Ediriweera
9.15 - 9.30 am	Synthesis and characterization of new heterocyclic compounds from the reaction of 4,7-dioxononanoic acid with 1,2-dinucleophiles	Chayanika padumadasa, Ajita M Abeysekara, and Nethmi De Alwis
9.30 - 9.45 am	Bioactivity of <i>Microcos paniculata</i> L leaf ethanolic extract: <i>In vitro</i> cholinesterase, protease enzyme inhibitory and anti oxidant activity	S P Samaradivakara and J K R R Samarasekara

9.45 - 10.00 am	Second derivative infrared spectroscopy used as a reliable tool to evaluate the functional authenticity of the interface of surface modified silica and nylon-6	Laleen Karunanayake and C J Narangoda
10.00 - 10.30 pm	TEA BREAK	
10.30 - 10.45 am	Study of the Pretreatment (Shodhana) of Roots of <i>Plumbago indica</i> L in Ayurveda	Chayanika padumadasa, A M Abeysekara, and Shalika Meedin
10.45 - 11.00 am	Decarboxylation of waste coconut oil for the production of Green Diesel	PH Gamage, U S K Weliwegama and H I C De Silva
11.00 - 11.15 am	Antidiabetic compounds in <i>Syzygium cumini</i> ready to serve herbal drink	PR D Perera, Sagarika Ekanayake and K K D S Ranaweera
11.15 - 11.30 am	Isolation and Characterization of probiotic " <i>Pediococcus acidilactici</i> " from Sri Lankan finger millet variety (<i>Elucine coracana</i>)	DM W D Divisekera, J K R R Samarasekara, C Hettiarachchi, J Goonaratne and S Gopalakrishnan
11.30 - 11.45 pm	<i>In vitro</i> starch digestibility and resistant starch content of selected banana varieties (<i>Musa species</i>) from Sri Lanka	R Sutharsana, S A S Jayawardena, J K R R Samarasekara and J Goonaratne

Abstracts of Research Papers to be presented at the 44th Annual Sessions 2015

Technical Sessions : A - 01

Selenium Content in Daily Meals Consumed by Sri Lankans - A preliminary study

K M S D Kiridena¹, D S M De Silva^{1*}, Sukumal Wimalasena¹, A T Kannangara¹ and H P Weerathna²

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²Department of Zoology, University of Kelaniya, Sri Lanka

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Selenium is a trace element which is essential to the human body as a micronutrient, mainly present as amino acid derivatives such as selenomethionine, selenocysteine and methylselenocystein. Selenium is beneficial but toxic in a narrow range (25 µg/day - 400 µg/day for person).¹ Selenium content in raw vegetables, cereal and legumes grown in Sri Lanka have been determined in previous studies.²⁻⁵

The study reports the selenium content of sun dried samples obtained from the plates of meals (rice and curries) consumed for lunch. Analysis was carried out on samples obtained from five districts. Determination of selenium was carried out using Hydride Generation Atomic Absorption Spectrometric method on acid digested samples. Statistical analysis was carried out using one way ANOVA and Tukey's pairwise comparisons in MINITAB Release 14. The range of mean selenium content in meals consumed by Sri Lankans in the five districts is 55-60 µg/kg with an overall mean of 56.67 ± 2.208 µg/kg. This value is comparable to the daily requirement, 55 µg/day given by Food and Nutrition Board, Institute of Medicine,

USA.

Concentration of selenium in fried chicken was found to be less than that in chicken curry. Analysis on selenium on different curries consumed by Sri Lankans [chicken curry, dhal curry and cooked green leaves (Mallum)] indicated that mean concentrations as 84.25 µg/kg, 51.41 µg/kg and 47.54 µg/kg respectively.

The present study revealed that intake of selenium per meal by Sri Lankans is in the range 55-60 µg/kg and there is no significant difference in selenium concentration in meals among the selected districts as well among individual households in each district.

Keywords: Selenium, Daily intake, Sri Lankan

Acknowledgement: Financial assistance by NSF Research Grant (RG/2010/AG/03)

References:

1. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC, (284-324)
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- Wimalasena, (2013), Selenium content in rice consumed by Sri Lankans, Chemistry in Sri Lanka, Institute of Chemistry Ceylon, 30(2): 42
3. B M S S Bandara, A T Kannangara, D S M De Silva and S Wimalasena, (2013), Selenium content in vegetables consumed by Sri Lankans, *Sri Lanka Association for the Advancement of Science Proceedings* 69, 159.
 4. P A Buwaneka, D S M De Silva, S Wimalasena and A T Kannangara, (2014) Determination of Selenium content in cereals and legume seeds grown in Sri Lanka, International Research Symposium on Postharvest Technology, Institute of Post Harvest Technology, Anuradhapura, Sri Lanka, pp. 27-32.
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Technical Sessions : A - 02

In vitro* 5-Lipoxygenase enzyme inhibitory and anti-oxidant activities of Selected Sri Lankan medicinal plant leaves: *Bacopamoni*eri, *Melaleucadendra* and *Sphaeranthusindicus

H D S M Perera and R Samarasekera*

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Inhibition of catalytic functions of 5-Lipoxygenase (5-LOX) enzyme to deplete biosynthesis of inflammatory mediators is considered as a promising therapeutic approach in the treatment of inflammatory diseases. Medicinal plants remain as potent sources of new 5-LOX inhibitors and antioxidants. *Bacopamoni*eri (Scrophulariaceae), *Melaleucadendra* (Myrtaceae) and *Sphaeranthusindicus* (Asteraceae) are some medicinal plants used in Ayurveda and traditional system of medicine for the treatment of many diseases, including inflammatory diseases. The objective of the present study is to investigate *in vitro* 5-Lipoxygenase related anti-inflammatory and antioxidant properties of ethanol extracts of leaves of *Bacopamoni*eri, *Melaleucadendra* and *Sphaeranthusindicus*.

Air-dried and powdered leaves of plants were extracted with ethanol using cold extraction technique. Anti-inflammatory activity of ethanol extracts was determined by 5-Lipoxygenase enzyme inhibitory assay. Anti-oxidant activities of three extracts were determined by DPPH free radical scavenging, Ferrous Ion Chelating (FIC), Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays. Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC) were determined using Folin-Ciocalteu (FC) and Aluminiumtrichloride methods respectively.

Melaleucadendra showed the highest 5-LOX inhibitory activity ($IC_{50}=48.71\pm1.15 \mu\text{g/mL}$) followed by *S. indicus* ($IC_{50}: 137.07\pm9.27 \mu\text{g/mL}$) and *B.monieri* ($IC_{50}: 346.56\pm5.58 \mu\text{g/mL}$) with comparison to Baicalein ($IC_{50}: 1.55\pm0.24 \mu\text{g/mL}$, $p<0.05$).

Sphaeranthusindicus showed the highest DPPH free radical scavenging activity ($IC_{50}: 109.57\pm0.24 \mu\text{g/mL}$) followed by *M.leucadendra* ($IC_{50}: 144.98 \pm 3.16 \mu\text{g/mL}$) and *B. monieri* ($IC_{50}=346.57\pm0.51 \mu\text{g/mL}$) in comparison to Trolox ($IC_{50}=5.29\pm0.09 \mu\text{g/mL}$, $p<0.05$). Showing the high hydrogen donating ability of three extracts as antioxidants. An effective metal chelating agent may provide protection against oxidative damage by inhibiting the production of reactive oxygen species and lipid peroxidation. The FIC activity of extracts of *B. monieri* ($IC_{50}=1829.44 \pm 122.21 \mu\text{g/mL}$), *M. leucadendra* and *S. indicus* (9.15 % and 20.15 % chelations at 1000 $\mu\text{g/mL}$) were found to be low in comparison to the reference standard EDTA-2Na ($IC_{50}=13.07\pm0.64 \mu\text{g/mL}$). In FRAP assay, extract of *B. monieri* showed the highest FRAP value (940.83 $\pm$ 112.73 mg Trolox equivalents (TE) /g) followed by *S. indicus* (332.56 $\pm$ 35.44 mg TE/g) and *M. leucadendra* (280.32 $\pm$ 5.87 mg TE/g), indicating the electron transfer ability, which may serve as a significant indicator of its potent antioxidant activity ($p<0.05$). The ORAC assay, which shows the peroxyl radical absorbance capacity of extracts, has been considered a preferred method for its biological relevance to the *in vivo* antioxidant efficacy. In this assay, the extract of *S. indicus* showed a promising ORAC (1031.75 $\pm$ 158.73 mg TE/g) in comparison to the standard Green Tea extract (1662.82 $\pm$ 0.22 mg TE/g) and moderate values have been recorded for *B. monieri* (650.79 $\pm$ 31.74 mg TE/g) and *M. leucadendra* (412.70 $\pm$ 83.99 mg TE/g) ($p<0.05$).

The highest TPC was recorded for *S. indicus* extract (49.36 $\pm$ 2.30 mg Gallic acid equivalents (GAE)

/g) followed by the extracts of *M. leucadendra* (26.37±1.44 mg GAE/g) and *B. monieri* (5.2±0.95 mg GAE/g). Highest TFC was recorded for *M. leucadendra* (11.49±0.37mg Quercetin equivalents (QE) /g), followed by *S. indicus* (3.98± 0.13 mg QE/g) and *B. monieri* (2.75±0.20 mg QE/g). Previous studies have shown that polyphenolic and flavonoid compounds are responsible for the reduction of oxidative stress due to antioxidant action. Hence, the higher TPC of the extract of *S. indicus* could be attributed to high DPPH radical scavenging activity and ORAC, whereas higher TFC of

the extract of *M. leucadendra* could be attributed to the higher 5-LEI activity.

The findings reveal that the ethanol extracts of leaves of above plants possess good 5-LOX related anti-inflammatory and anti-oxidant properties. The ethanol extracts of leaves of *M. leucadendra* can be considered as a good source of 5-LOX enzyme inhibitors, which is supported by good anti-oxidant activity, TPC and TFC.

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Isolation and molecular characterization of Sri Lankan *Bacillus thuringiensis* with potential Lepidopteran activity

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Lepidoptera is a detrimental pest of cruciferous crops worldwide. Pest attack is a major threat for vegetables and rice crops in Sri Lanka, which can lose 15-20% crop loss every year. *Bacillus thuringiensis* (*Bt*) is an aerobic, Gram-positive, rod-shaped and endospore forming bacterium belongs to family Bacillaceae. The *Bt* bacterium produces insecticidal proteins such as crystal (*Cry*), cytolytic (*Cyt*) and vegetative (*Vip*) proteins. Different strains of *Bt* produce more than 65 different, but related, insecticidal crystal proteins (ICP) known as δ -endotoxins and are the pre dominant type among *Bt* insecticidal proteins. These proteins are toxic to larvae of different insect orders including Diptera, Lepidoptera, Coleoptera, Hemiptera, Hymenoptera, Homoptera, Othoptera, nematodes, mites and protozoa. *Cry1*, *Cry2* and *Cry9* proteins show strongest toxicity to Lepidopterans. The objective of the present study is the isolation and molecular characterization of Sri Lankan *Bt* with potential Lepidopteran activity.

In this study, *Bt* were isolated from soil samples collected from Anuradhapura, Mathale, Puttalam, Rathnapura, Kadawatha, Matara, Nihiluwa, Thihagoda, Makandura, Lunugala, Wariyapola and Malimbada. *Bt* were isolated from soil, based on acetate selection/ heat treatment method. Isolated *Bt* were grown on *Bacillus* agar to differentiate *Bt* like colonies depend on colony colour and morphology. Crystal violet and Coomassie blue stainings were carried out to detect the presence of parasporal crystals and endospore staining was used to examine the presence of endospores. Since *Cry* genes are mainly resides in megaplasmids of *Bt*, plasmid DNA were extracted, using an optimized protocol, from overnight grown *Bt*

culture in Luria Bertini broth. Presence of Lepidoptera specific *Cry* genes; *Cry1*, *Cry2* and *Cry9* were investigated by PCR analysis using both universal and gene specific primers with an optimized Polymerase Chain Reaction (PCR) conditions.

Use of Chromogenic *Bacillus* agar allowed easy identification of *Bt* colonies from the rest as it differentiate *Bacilli* species with specific colony colors. Blue or blue/green colonies with irregular margins are suspected as *Bt* colonies. This chromogenic method significantly narrows down the spectrum for selection of *Bt* like isolates. The presumptive isolates upon Coomassie Blue staining and crystal violet staining revealed the presence of parasporal crystal inclusions, which further confirmed the isolates as *Bt*. Further, endospores were stained as green elliptical structures within pink vegetative cells in all *Bt* like colonies. Chromogenic and phenotypic characterizations together confirmed 18 isolates as *Bt*.

The PCR amplification analysis of those 18 *Bt* isolates revealed the presence of amplified fragments characteristic of Lepidopteran toxic *Cry* genes; *Cry1*, *Cry2* and *Cry9* in 7 *Bt* isolates. Among the screened 18 *Bt* isolates, two *Bt* isolates; AB17 and AB22 contained all 3 *Cry* genes while *Bt* isolates; AB6, AB7 and AB10 contained *Cry1* and *Cry9* genes. *Bt* isolates; AB15 and AB16 contained *Cry1* and *Cry2* genes. In conclusion, the results suggest that these seven *Bt* isolates, possess insecticidal Lepidopteran active *Cry* genes; *Cry1*, *Cry2* and *Cry9*. Therefore these *Bt* isolates have potential to be used as biological controlling agents against Lepidoptera insects.

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Elastase , tyrosinase inhibitory and antioxidant activity of *Rubia cordifolia* root extract

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Reactive oxygen species (ROS) and free transition metal ions cause oxidative damage to various biomolecules. Although the skin has self-defense system to deal with ROS, excessive and chronic exposure to UV can overwhelm the condition leading to oxidative stress and damage resulting premature aging. In normal condition, skin produces enzymes such elastase and collagenase, at similar rate as aging process occurs and age increases. However, with over exposure to sunlight (UVA and UVB), the presence of excessive ROS and smoking habit, the enzymes are produced at a faster rate resulting in faster degradation of elastin and collagen, which are the main foundation of extracellular matrix (ECM) of the dermis. Additionally, excessive exposure to sunlight, induce production of melanin in the skin layer and tyrosinase is the responsible enzyme that initiates skin pigmentation and melanin production.

Rubia cordifolia (Wal Madata, Rubiaceae) is widely used as an effective blood detoxifier, to treat skin disorders like hyper pigmentation, scabies, acne and allergies. The herb used in the treatment of liver diseases, gall stones and amenorrhea. The objective of the present study was to determine the elastase inhibitory, tyrosinase inhibitory and antioxidant activity of the root extract of *R. cordifolia*, which is an ingredient in cosmetic formulations.

Air-dried and powdered plant roots were extracted with ethanol following a cold extraction protocol. Plant extracts were evaluated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging, Ferric Ion Antioxidant Potential (FRAP) activity and Oxygen Radical Absorbance Capacity (ORAC) assays. Total phenolic content (TPC) was determined using Folin-Ciocalteu method. Extract was also evaluated *in vitro* by tyrosinase inhibitory and elastase inhibitory activity.

Ethanol extract of roots of *R. cordifolia* exhibited DPPH free radical scavenging activity having IC_{50} value of $84.7 \pm 2.06 \mu\text{g/mL}$ which was less than green

tea extract and trolox ($p < 0.05$). ORAC assay was conducted to evaluate the peroxy radical absorbance capacity of *R. cordifolia* *in vitro* and the extract gave an ORAC value of $1501 \pm 63.67 \text{ mg Trolox Equivalent /g}$ extract, which was comparable to that of green tea extract.

Using FRAP assay the ability of the extract to deviate the mechanism of Fenton reaction by chelating metal ions such as Fe^{2+} and Cu^{2+} , which are responsible to convert the hydrogen peroxide to hydroxyl radical on the skin can be measured. Ethanol extract of *R. cordifolia* showed a good FRAP value which was $953.33 \pm 6.80 \text{ mg TE/g}$ of extract.

Ethanol extract of roots of *R. cordifolia* showed good TPC value of $61.47 \pm 2.23 \text{ mg Galic acid Equivalent /g}$ of extract which indicates phenolic compounds have the ability to destroy radicals hence, they possess good antioxidant activity.

Rubia cordifolia showed moderate elastase inhibitory activity having 17.18% inhibition at $500 \mu\text{g/mL}$ with comparison to that of positive control, quercetin ($IC_{50} 221.69 \pm 5.52 \mu\text{g/mL}$). However, because of the colour interference it was not possible to test higher concentrations of *R. cordifolia* extract to evaluate IC_{50} values. Root extracts of *R. cordifolia* showed a moderate tyrosinase inhibitory activity having 20.94% inhibition at $500 \mu\text{g/mL}$ which was less than ascorbic acid ($IC_{50} 69.33 \pm 2.56 \mu\text{g/mL}$). However *R. cordifolia* extract is are widely used in skin whitening formulations. Other biological assays are required to evaluate the potential skin whitening properties of *R. cordifolia* extracts.

High antioxidant activity and moderate elastase and tyrosinase inhibitory activities were detected for the root extract of *R. cordifolia*. Further bioactivity studies are required to assess cosmetic properties of *R. cordifolia* extracts.

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Biochemical and molecular characterisation of probiotics from fermented traditional rice varieties

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Rice, being the staple diet of Sri Lanka, is an important crop that occupies 34% of cultivated area. Rice provides 45% of total calories and 40% of total protein for the average Sri Lankan. Traditionally, cooked rice is left to ferment overnight and, along with added coconut milk and other condiments, is consumed for breakfast, which is called *Diyabath*. *Diyabath* has healing properties for common stomach ailments such as gastritis and diarrhoea, which are caused by pathogenic microorganisms. Rice samples used in this study were obtained from the Rice Research Institute of Sri Lanka at Bathalagoda.

The objective of this study is to characterise, through biochemical and molecular techniques, the potentially probiotic strains of bacteria from fermented traditional rice varieties such as Madathawaalu, Pachchaperumaal, Suduheenati, Suwandel and Kuruluthuda.

The selected rice varieties were fermented and cultured on deMan, Rogosa and Sharpe agar that selectively facilitates the growth of gram positive bacteria. The isolates were subjected to morphological and biochemical tests. Thereafter, bile, pH, salt and temperature tolerance tests were carried out to identify the probiotic potential of the isolated bacteria. DNA was extracted from pure cultures of each isolate and the 16S ribosomal RNA gene was amplified by Polymerase Chain Reaction using universal bacterial 16S rRNA region primers 27F and 1492R. The variable regions 1-5 of the 16S rRNA gene were amplified using nested PCR technique with primers 27F and WLAB2R. The analysis of variable regions 1, 2 and 3, in 16S rRNA gene sequence, aids in the accurate characterisation and identification of bacteria¹. The amplified fragments were purified and sequenced using Big Dye Terminator Cycle Sequencing kit and Applied Biosystems 3500 Genetic Analyzer. The resultant sequences were searched over the GenBank database using the BLAST tool.

A total of nine isolates were obtained from Pachchaperumaal, Suduheenati, Suwandel and Madathawaalu. The colonies of these isolates were circular with irregular edges and possessed a mucoid texture. All nine isolates were identified as being Gram positive rods and cocci according to the Gram's test. However, when further analysis for probiotic potential was conducted, only four isolates displayed tolerance under gastrointestinal physiological conditions of acid, bile, salt and temperature. These four tolerant isolates were CSd1, CSd2, CSw1 and Cp2.

The data obtained from the sequence analysis indicated that all four of the isolates were *Bacillus subtilis*. This bacteria is a spore former that is capable of withstanding the harsh intestinal conditions^{2,3}. This bacterium is currently marketed commercially, as an oral probiotic supplement for human use⁴. It is recommended that further studies should be carried out on these isolates to confirm the bacteria up to sub species level and assess its potential to be used as a probiotic supplement for human use.

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Chemical and microbiological analysis of toothpaste available in leading supermarkets in Sri Lanka

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Toothpaste is a substance used by all humans two to three times daily for the purpose of cleaning the accessible surfaces of teeth and to provide oral hygiene. Apart from water, toothpastes contain a variety of components, the three important ones being abrasives (origin of heavy metals), fluoride, and organic compounds (surfactants/ flavors/ sweeteners/ binding agents/ preservatives). The analysis of toothpaste available in the Sri Lankan market, for the presence of heavy metals, organic compounds, fluoride levels and microbes and to compare the results with the tolerable limitations recommended by the Sri Lanka Standards Institution¹ are the main objectives of the study. Eight brands (6 local and 2 imported) were chosen and five from each brand were collected by random sampling method. No previous work related to the analysis of toothpaste in Sri Lanka has been reported.

Heavy metals which enter the toothpaste via abrasives are detrimental when ingested above tolerance levels and daily use significantly affect the human health. Toxicological guidance values recommended for heavy metals by Sri Lanka Standards Institution (SLSI) for toothpaste¹ are 1 mg/kg for arsenic and 1 mg/kg for lead. For other metals, limits have not been developed. The limits recommended by SLSI for fluoride ion in toothpaste is in the range 850 – 1150 mg/kg. When considering the nature of the toothpaste, it promotes a suitable environment to microorganisms to grow and create product spoilage and health risks to humans. Therefore one of the important parameters when assessing the quality of a toothpaste is its bacteriological property. As per Sri Lanka standard microbial limits for toothpaste are as follows: total aerobic bacteria per gram, maximum 1000 cfu, *Escherichia coli* per 10g and *Salmonella* per 10g should be absent. There are many organic compounds present in toothpaste. They are added deliberately to enhance the quality of toothpaste. However, they can be toxic when taken in large amounts.

Heavy metals in toothpaste were analyzed using

atomic absorption spectrometry (GFAAS/FAAS). All brands tested had detectable amounts of heavy metals. The results are as follows: cadmium 0.02 – 0.29 mg/kg, arsenic 0.02 – 2.98 mg/kg, lead 0.20 – 2.64 mg/kg, nickel 0.20 – 1.37 mg/kg, copper 0.43 – 3.36 mg/kg and zinc 5.94 – 13.52 mg/kg. As and Pb were crucial for some brands. Fluoride levels were determined by analyzing aqueous extracts of toothpastes using fluoride ion selective electrode with suitable calibration. The results indicate that fluoride levels vary widely, 110-1550 mg/kg. Among the eight brands, two brands were free from added fluoride. Three brands contained fluoride levels above the limit, 1190-1525 mg/kg and the remaining brands were below the limit, 110-490 mg/kg. Microbial contamination was very low in selected brands due to the presence of preservatives. In all eight brands, aerobic bacteria (<10) and *Salmonella* was not detected. However, *Escherichia coli* were present in two brands. Organic compounds present in toothpaste were determined qualitatively, having extracted to methylene chloride and analyzed using GCMS. Results revealed a wide range of organic chemicals which are too numerous to specify. However, it should be mentioned that some toxic organic compounds such as butyl paraben and benzene were present in some brands, which may have significant effect on human health depending on the amount.

Finally, it can be concluded based on the preliminary data available that it is high time more effort was made to determine and limit the presence of heavy metals, fluoride, organic compounds quantitatively along with control of microbial contamination in toothpastes of all brands available in Sri Lanka. This should be of high priority and will be a subject of future investigation.

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Synthesis of some Cu(I) complexes with bidentate N and P donors

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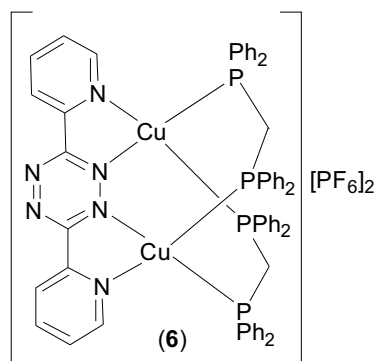
Molecular and supramolecular architectures containing Cu(I) centres are known to exhibit photo- and electro-luminescence. Electron transfer reactions involving Cu(II)/(I) centers have attracted attention of many researchers as these reactions are strongly related to the biologically important catalytic processes. The interest in Cu(I) complexes has risen recently as an alternative to other more expensive light harvesting complexes of transition metals such as ruthenium and iridium. Cu(I) complexes of the type $[\text{Cu}(\text{N}^{\wedge}\text{N})(\text{P}^{\wedge}\text{P})]^+$ have shown unusually efficient, long-lived photoluminescence. It is of interest to explore the synthetic routes to Cu(I) complexes with bidentate ($\text{N}^{\wedge}\text{N}$), ($\text{P}^{\wedge}\text{P}$), and mixed ($\text{N}^{\wedge}\text{N}$) and ($\text{P}^{\wedge}\text{P}$) donors. In this communication, we report the preliminary studies carried out to prepare a series of Cu(I) complexes, including a binuclear complex with bridging ($\text{N}^{\wedge}\text{N}$) and ($\text{P}^{\wedge}\text{P}$) donors.

The reaction of Cu(I) salt $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ with 2 equivalents of 6,6'-dimethyl-2,2'-bipyridine (6,6'-Me₂bpy) gave $[\text{Cu}(6,6'\text{-Me}_2\text{bpy})_2]\text{PF}_6$ (**1**) in 81% yield. This complex and other complexes are characterized by IR, Mass and NMR spectroscopy. The ¹H-NMR spectrum of (**1**) showed two doublets and a triplet for the pyridyl moiety whilst the methyl protons appeared as a singlet at 2.24 ppm. Treatment of 2 equivalents of 4,5-bis(diphenylphosphino)-9,9'-dimethyl xanthene (Xantphos) with $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ gave the $[\text{Cu}(\text{Xantphos})_2]\text{PF}_6$ (**2**) in 86% yield. The ³¹P-NMR spectrum of (**2**) showed a broad singlet at -17.6 ppm.

The $[\text{Cu}(6,6'\text{-Me}_2\text{bpy})(\text{Xantphos})]\text{PF}_6$ (**3**) was prepared by treating $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ with a mixture of 6,6'-Me₂bpy and Xantphos in (1:1) ratio and it isolated as a yellow solid in 88% yield. The ³¹P-NMR spectrum of (**3**) showed a singlet at -11.7 ppm. Treatment of $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ with one equivalent Xantphos in acetonitrile gave a white solid in 64% yield. It showed a phosphorus-31 resonance at -12.9 ppm. Characterizing data including elemental analysis suggests the complex (**4**) to be with the composition $[\text{Cu}(\text{NCMe})(\text{Xantphos})]\text{PF}_6$. Treatment of this Cu(I) complex (**4**) with one equivalent of 6,6'-Me₂bpy gave the mixed-ligand complex $[\text{Cu}(6,6'\text{-Me}_2\text{bpy})(\text{Xantphos})]\text{PF}_6$ (**3**) in 96% yield.

Moreover, we studied the chemistry of $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ with the diphosphine,

bis(diphenylphosphino) methane (dppm) which is known to bridge two metal centres than forming 4-membered chelate rings. Treatment of $[\text{Cu}(\text{NCMe})_4]\text{PF}_6$ with one equivalent of dppm in acetonitrile resulted in the formation of a white solid in 86% yield. It showed a phosphorus-31 resonance at -6.4 ppm. The proton resonances at 3.53 (multiplet) and 2.19 (singlet) ppm are assigned to the CH₂ groups and coordinated acetonitrile ligands. We tentatively suggest this complex to be $[\text{Cu}(\text{NCMe})_2(\mu\text{-dppm})_2\text{Cu}(\text{NCMe})_2][\text{PF}_6]_2$ (**5**) with two dppm ligands bridging two Cu(I) centres. Replacement of all four acetonitrile ligands in (**5**) with a ligand containing four nitrogen donor atoms was studied, thus, we reacted (**5**) with one equivalent of 3,6-di(2-pyridyl) tetrazine (dptz) which has the capability to accommodate two metal centres. Treatment of complex (**5**) with one equivalent of dptz gave dark purple crystals of $[\text{Cu}_2(\mu\text{-dptz})(\mu\text{-dppm})_2][\text{PF}_6]_2$ (**6**) in good yield. The ¹H-NMR spectrum of (**6**) showed peaks at 9.26, 8.29, 8.23 and 7.53 ppm for the protons of dptz ligand. The CH₂ protons of dppm are not now chemically equivalent and appeared as two broad multiplets at 3.94 and 3.44 ppm.



In conclusion, we have developed synthetic routes to Cu(I) complexes of the type $[\text{Cu}(\text{N}^{\wedge}\text{N})_2]^+$, $[\text{Cu}(\text{P}^{\wedge}\text{P})_2]^+$ and $[\text{Cu}(\text{N}^{\wedge}\text{N})(\text{P}^{\wedge}\text{P})]^+$. We also prepared a binuclear Cu(I) complex containing bridging 3,6-di(2-pyridyl) tetrazine and dppm ligands.

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Synthesis and biological studies of *fac*-[ReL(CO)₃]BF₄; L=N(SO₂piperidinyl)dipicolylamine

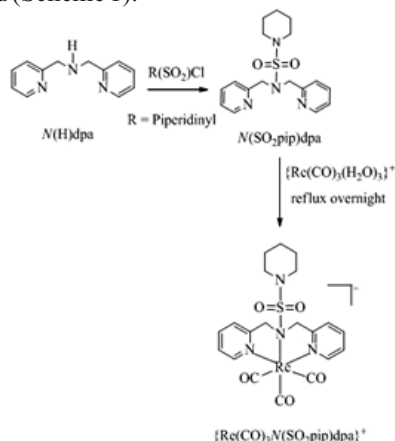
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Organometallic complexes containing tertiary sulfonamide nitrogen-to-metal complexes of normal bond length are scarcely found in the history of synthetic inorganic chemistry. Organometallic complexes may be used either as diagnostic agents or as therapeutic agents in medicine. Pharmaceuticals consisting of organometallic complexes are widely used for cancer therapy. This is done by judiciously selecting suitable metals and ligands to create novel complexes. Our choice was to incorporate a piperidinyl group which falls into the category of exogenous ligands which, when labeled with suitable radioisotopes have been reported as potential radiopharmaceuticals for imaging of sigma receptors. During this study, a novel ligand (L = (N(SO₂pip)dpa)) incorporating a central piperidinyl group and its corresponding Re complex ([Re(CO)₃(N(SO₂pip)dpa)]⁺) have been synthesized in good yield (Scheme 1).



Scheme 1. Synthetic route for N(SO₂pip)dpa and [Re(CO)₃(N(SO₂pip)dpa)]⁺

The methylene CH₂ signal seen as a singlet (4.54 ppm) in a spectrum of the ligand, appears as two doublets (5.39, 5.01 ppm) in a ¹H NMR spectrum of the [Re(CO)₃(N(SO₂pip)dpa)]⁺ complex (Figure 1) and confirms the presence of magnetically non equivalent protons upon coordination to Re. Structural results revealed that the Re—N bond lengths fall within the normal range establishing the coordination to metal. The presence of intra-ligand π→π* and n→π* transitions are indicated by the absorption peaks around 200-250 nm in UV-Visible spectra. An absorption peak at 325 nm in a UV-Visible spectrum of the metal

complex was identified as a MLCT transition. The S—N stretch observed as a strong peak at 923 cm⁻¹ for N(SO₂pip)dpa, shifts into shorter frequency, at 830 cm⁻¹ in an FTIR spectrum of the [Re(CO)₃(N(SO₂pip)dpa)]⁺ corroborating, the direct coordination of sulfonamide nitrogen to Re. This novel ligand display intense fluorescence in a fluorescence spectrum. The metal complex, although fluorescent, shows lower intensity than the ligand indicating quenching of fluorescence upon coordination to Re.

Mammalian cell toxicity of N(SO₂pip)dpa (L) and [Re(CO)₃(N(SO₂pip)dpa)]⁺ (C) was assessed with Trypan Blue dye exclusion assay on murine peritoneal cells. Although N(SO₂pip)dpa shows no toxicity at levels tested, [Re(CO)₃(N(SO₂pip)dpa)]⁺ shows acute cytotoxicity with an IC₅₀ of 889.6 μM. Relatively low IC₅₀ values given by human breast cancer cells MCF-7 (L1 = 139 μM, C1 = 360 μM) indicate that L1 and C1 are promising novel compounds that can be further investigated on their usage as potential anti-cancer agents and cancer cell imaging agents.

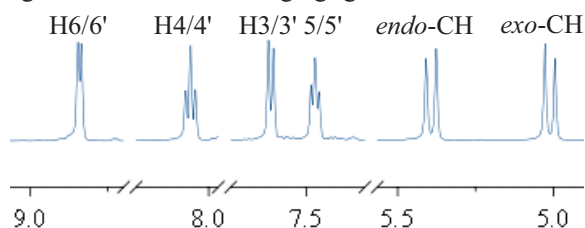


Figure 1. ¹H NMR spectrum of the [Re(CO)₃(N(SO₂pip)dpa)]⁺ complex in DMSO-d₆

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Nitric oxide scavenging activity of the herbal formulation Nawarathne Kalka used in traditional medicinal systems in Sri Lanka for the treatment of rheumatoid arthritis

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Nawarathne Kalka (NK) together with different adjuvant or vehicles (*Anupana*) has been excessively used in Ayurveda and Traditional Medicinal systems in Sri Lanka for the treatment of various ailments for more than hundreds of years. NK is mainly used as anti-inflammatory and immune enhancing drug for arthritic conditions¹. The objective of this study was to evaluate the *in vitro* nitric oxide (NO) scavenging potential of NK to express the role of NK in arthritic conditions. NO is implicated in many different disease states such as septic shock, hypertension, stroke, cancer, AIDS, Alzheimer's disease, arthritis, and neurodegenerative diseases. DNA fragmentation, neuronal cell death and cell death can occur as a result of excess NO. Additionally NO is unstable in aerobic conditions producing reactive intermediates.

NK was purchased from a traditional medicinal drug store. Contents of three sachets were pooled together and a wet weight of 15 g was refluxed with 400 ml of deionized water for 3 hours. The extract was filtered using Whatman no.1 filter paper and the filtrate (1.0ml) was used for each experiment. Scavenging of NO was evaluated under a NO generating source, aqueous sodium nitroprusside, at physiological pH according to a previously published method². The released nitric oxide interacts with oxygen to produce nitrite ions. Nitrite ions were then diazotized with sulfanilamide and naphthylethylenediamine dihydrochloride (NED), and absorbance was measured spectrophotometrically at 540 nm. The presence of NO scavengers decreases the production of this chromophore. In this study the released NO radicals were scavenged by a series of NK concentrations (658.0 - 10.3 µg/ml) and the positive control, ascorbic acid (33.3 - 4.2 µg/ml). The percentage inhibition (%I) was calculated as:

$$\%I = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

A sample where NK extract/ascorbic acid was replaced by deionized water was used as the negative control. The concentration required to inhibit 50% of

nitric oxide generated respective to the control (EC_{50}) was calculated from the dose response curves plotted as %I versus concentration for both NK and ascorbic acid (Figure 1).

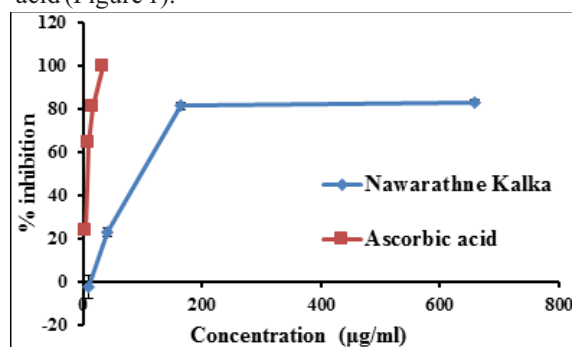


Figure 1: Dose response curves for % inhibition of nitric oxide by NK and ascorbic acid

A concentration dependent scavenging of NO radicals was observed for both NK and ascorbic acid. However, the scavenging activity of water extract of NK ($EC_{50} = 99.3 \pm 8.4$ µg/ml) is lesser than ascorbic acid ($EC_{50} = 7.3 \pm 0.3$ µg/ml).

Water soluble phytochemicals present in NK exerts a strong effect on scavenging of NO radicals which is an important phenomena required to control inflammatory responses during arthritic conditions. The activity of non water soluble compounds present in NK needs to be evaluated.

Keywords: Anti-inflammatory, arthritis, *Nawarathne Kalka*, NO scavenging

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Antioxidant and Cytotoxic Activities of Proanthocyanidins of the Bark of *Thespesia populnea* (L.)

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Thespesia populnea is a tree that belongs to the family Malvaceae. In Sri Lanka it is commonly known as Gan-suriya. Almost all the parts of the tree have been utilized traditionally as medicine to treat skin and liver diseases, hemorrhoids, diarrhea, etc. Additionally, in Sri Lanka the bark of the tree is been utilized for the treatment of cancers. The phytochemical analysis of the aqueous ethanolic extract of the bark revealed the presence of flavonoids, saponins, sterols and alkaloids and the absence of anthraquinones in accordance with published data. Most importantly this study revealed the presence of proanthocyanidins, which has not been reported before. Ethyl acetate and aqueous soluble proanthocyanidin fractions (EASPA and AQSPA respectively) were extracted according to a previously published method with minor modifications. They were purified by chromatography on Sephadex LH-20. Acid catalyzed cleavage and Prussian blue tests revealed that proanthocyanidins have been successfully separated from other phenolics. The yields of purified EASPA and AQSPA fractions were 0.04% and 0.64% (by weight) of the fresh bark. Acid catalyzed cleavage followed by TLC studies of both EASPA and AQSPA fractions alongside anthocyanidin working standards, cyanidin, delphinidin and pelargonidin isolated from pomegranate arils under acidic conditions showed the presence of cyanidin and delphinidin, suggesting that they are composed of (epi)catechin and (epi)gallocatechin units with (epi)catechin being more abundant compared to the other.

The preliminary antioxidant activity of purified EASPA and AQSPA fractions were determined using the DPPH assay according a previously published method with some modifications. According to the DPPH assay the IC_{50} values of EASPA and AQSPA fractions were 0.0725 mg/mL and 0.0781 mg/mL respectively and that of ascorbic acid was 0.125 mg/mL. Ascorbic acid is an established standard used for antioxidant studies. The IC_{50} values of the

proanthocyanidin samples are clearly lower than the standard. Therefore, samples possess higher antioxidant capacity than ascorbic acid. In addition, the cytotoxic effect of purified EASPA and AQSPA fractions against MCF 7 cell line was determined using the Sulphorhodamine B (SRB) assay according to a previously reported method. According to this study both EASPA and AQSPA fractions exhibited cytotoxic activity. For EASPA and AQSPA fractions the IC_{50} values after 24 hours were 266.8 μ g/mL and 186.1 μ g/mL while after 48 hours they were 150.0 μ g/mL and 150.8 μ g/mL respectively.

Conclusion

For the first time we report the presence of proanthocyanidins in the bark of *Thespesia populnea*. Proanthocyanidins have been successfully extracted and purified from other phenolics. They consist of (epi)catechin and (epi)gallocatechin monomeric units and possess antioxidant and cytotoxic activities.

Acknowledgement

We thank the Institute of Biochemistry, Molecular Biology and Biotechnology for carrying out cytotoxicity studies.

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Synthesis and characterization of new heterocyclic compounds from the reaction of 4,7-dioxononanoic acid with 1,2-dinucleophiles

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4,7-Dioxocarboxylic acids have been known for a very long time, however, it was only in 1998 that their spectroscopic data were reported for the first time.^{1,2} The chemistry of 4,7-dioxocarboxylic acids has not been explored in detail. These acids can be easily synthesized from furfural, which is a readily available, cheap and versatile organic compound that can be derived from a variety of agricultural byproducts.³ They are potentially good precursors for the synthesis of 5- and 6- membered heterocyclic compounds with pharmaceutical interest due to the presence of two keto carbonyl groups in a 1,4-relationship as well as a carboxyl carbonyl group and keto carbonyl group in a 1,4-relationship.⁴ We have already reported the reaction of 4,7-dioxononanoic acid with hydrazine and here we report the synthesis and characterization of two new heterocyclic compounds (oxazine derivative and a pyrrole derivative) from the reactions of 4,7-dioxononanoic acid with dinucleophiles, phenyl hydrazine and hydroxylamine.⁵

Reactions of 4,7-dioxononanoic acid with hydroxylamine and phenyl hydrazine are shown in Figure 1.

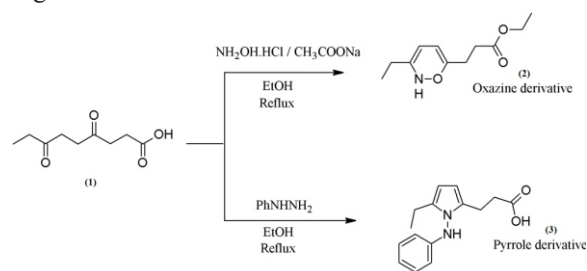


Figure 1: Reactions of 4,7-dioxononanoic acid with hydroxylamine and phenyl hydrazine

The major product (2) from the reaction between 4,7-dioxononanoic acid and hydroxylamine showed a single peak in the gas chromatogram and the corresponding mass spectrum showed the molecular ion at 211.1. The resulting strong fragment at 124.0 corresponded to $(M-CH_2CO_2Et)^+$. The IR spectrum of compound (2) showed the ester C=O stretching at

1731.991 cm^{-1} , N-H stretching at 3348.87 cm^{-1} and N-O stretchings at 1463.30 cm^{-1} and 1374.40 cm^{-1} confirming the assigned structure. The UV absorbances of compound (2) at 214 and 322 nm were in accordance with a typical oxazine derivative. Similarly the major product (3) from the reaction between 4,7-dioxononanoic acid and phenyl hydrazine showed a single peak in the gas chromatogram and the corresponding mass spectrum with the molecular ion at 258.1. The resulting fragments at 199.1 and 93.0 corresponded to $(M-CH_2COOH)^+$ and $(NHC_6H_5)^+$ respectively. The IR spectrum of compound (3) showed the carbonyl stretching of the carboxylic acid at 1707.75 cm^{-1} , N-H stretching at 3324.72 cm^{-1} , C=C aromatic ring stretching at 1602.00 cm^{-1} and 1496.22 cm^{-1} confirming the assigned structure. The OH stretching of the carboxylic acid was not observed, however, TLC using bromocresol green as the indicator, which is specific for carboxylic acid groups, confirmed its presence. The UV absorbances of compound (3) at 244 and 280 nm were in accordance with that of a pyrrole derivative.

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Bioactivity of *Microcos paniculata* L. leaf ethanolic extract: *In vitro* cholinesterase, protease enzyme inhibitory and antioxidant activities

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Cancer and neurodegenerative Alzheimer's disease (AD) are diseases, which have become a global public health concern. Enzymes and free radicals play key roles in many pathological disorders. Therefore, plant-derived multi target therapeutic agents with enzyme inhibitory and antioxidant activities are currently being investigated as viable means of such disease management. *Microcos paniculata* L. (Malvaceae) is commonly called "Kohu Kirilla" in Sri Lanka, and is used in traditional systems of medicine in the Asian region. However, cholinesterase and protease inhibitory activity of this plant has not been studied. The objective of the present work was to evaluate the total ethanolic leaf extract of *M. paniculata* for its cholinesterase (ChE), protease inhibitory and antioxidant activities and the total phenolic and flavonoid content.

Air-dried and powdered plant material was extracted with ethanol by cold extraction technique. Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), -chymotrypsin and elastase enzyme inhibitory activities of *M. paniculata* extract were measured. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Ferrous Iron Chelating (FIC), Ferric Reducing Antioxidant Potential (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays. Total phenolic content (TPC) and total flavonoid content (TFC) were determined by Folin-Ciocalteu and AlCl₃ methods respectively. All assays were carried out in triplicates using Spectra Max 96 well micro plate reader.

The ethanolic leaf extract of *M. paniculata* showed moderate AChE inhibitory activity of IC₅₀ 131.90 ± 2.02 µg/mL in comparison to Galanthamine, a clinical inhibitor (IC₅₀ 0.58 ± 0.00 µg/mL) and a 36.87% inhibition at 500 µg/mL was recorded for the extract's BChE inhibitory activity in comparison to Galanthamine, IC₅₀ 3.99 ± 0.25 µg/mL. However protease inhibitory activity of the extract against -

chymotrypsin and elastase enzymes were recorded as 42.81% and 21.62% inhibition respectively at 500 µg/mL compared to the positive standards, Chymostatin (IC₅₀ 5.93 ± 0.10 µg/mL) and Quercetin (IC₅₀ 221.69 ± 5.52 µg/mL). Leaf extract exhibited marked DPPH radical scavenging activity (IC₅₀ 44.60 ± 1.17) in comparison to Trolox (IC₅₀ 4.6 ± 0.0 µg/mL). Lower ferrous ion chelating activity was indicated with an IC₅₀ value of 1686.10 ± 30.87 µg/mL in comparison to EDTA (12.74 ± 0.21 µg/mL). Moderate reducing power was exhibited with a FRAP value of 715.00 ± 4.45 mg Trolox Equivalent/gram (mg TE/g) of extract. The leaf extract exhibited ORAC of 280.08 ± 0.28 mg TE/g of extract in comparison to green tea (IC₅₀ 1362.82 ± 0.22 mg TE/g of extract) and TPC and TFC was found to be 79.65 ± 0.66 Gallic acid equivalent/g of extract and 47.08 ± 0.45 Quercetin equivalent/g of extract, respectively. *M. paniculata* antioxidant and acetylcholinesterase inhibitory bioactivity could be attributed to the presence of phenols and flavonoids in the plant but also could be due to the activity of other secondary biomolecules present in the extract.

The results indicate the ethanolic leaf extract of *M. paniculata* possesses good antioxidant and acetylcholinesterase inhibitory activity and low protease inhibitory activity. From these results, we conclude that the ethanolic extract of *M. paniculata* leaf might have potential as a source of therapeutically active compounds, which could serve as chemical templates for the design of an effective and safe anti cancer or anti AD drug. These preliminary results provide a scientific basis for further bioassay-guided characterization of bioactive plant metabolites from this plant. This is the first report of cholinesterase, -chymotrypsin and elastase inhibitory activities of *M. paniculata*.

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Second derivative infrared spectroscopy used as a reliable tool to evaluate the functional authenticity of the interface of surface modified silica and nylon-6

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In the present study, surface modified silica was produced by reacting silica with various weight percentages of gamma-aminopropyltriethoxysilane (g-APS). Then surface modified silica was incorporated to nylon-6 by injection molding to prepare composite samples. Surface modified silica was subjected to FTIR analysis in order to identify the surface functionality. Composite samples were acid etched by formic acid and the residual silica was also subjected to FTIR analysis to identify the interfacial structure of nylon-6 (PA-6) and surface modified silica. Here, Fourier self deconvolution (FSD) and 2nd derivative process of FTIR spectroscopy were used since they have provided reliable spectral analysis and structural integrity in previous studies¹⁻³. Interfacial arrangement of surface grafted PA-6 chains to the surface modified silica surface has been evaluated with the help of protein secondary structure⁴. Throughout the current research, 2nd derivation of FTIR absorption spectra was used to enhance the resolution of the overlapped peaks and to understand the peak maximum. Peak identification was further improved by coupling 2nd derivation with FTIR spectral subtraction when analyzing the surface coating of the modified silica. Peak positions of the stretching vibrations of silanol (Si-OH) groups which appear as a shoulder to Si-O-Si asymmetric stretching vibrations were successfully identified through 2nd Derivation and FSD process. Since subtracted spectra contains high amount of weak intense noise peaks, all peak positions were exactly located by the 2nd derivation of the FTIR spectra. FTIR spectral results of modified silica suggest that g-APS was successfully attached to the surface of silica.

FTIR spectra of the acid etched samples demonstrate that PA-6 chains were chemically bonded

on to the surface of silica. 2nd derivation spectra evidently show the occurrence of the peak shift of the individual peak positions of acid etched samples from pure nylon-6. The Second derivation spectra of the Amide II finger band was not distorted in PA-6 grafted samples contrast with pure PA-6. It confirms that peak shift only exist in Amide A band and Amide I finger band regions. Throughout the experiment, the 2nd derivation of the FTIR spectra and FSD process were successfully used to identify the exact peak locations of the unresolved spectral features and shoulder peaks which increased the authenticity of the spectral data.

Acknowledgement Authors wish to thank Elastomeric Pvt. Ltd. for their technical support.

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Study of the Pretreatment (Shodhana) of Roots of *Plumbago indica* L. in Ayurveda

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Plumbago indica L. (Plumbaginaceae) is a medicinal herb, credited with vast number of potential therapeutic properties which is heavily used in traditional and ayurvedic medicinal systems in Sri Lanka and India. Naphthoquinones are the major secondary metabolites in the roots, of which plumbagin, a volatile compound is predominant. Ayurveda formulations are incorporated with air-dried roots of *P.indica* L. upon subjecting to a pretreatment with lime water and this pretreatment is called "Shodhana". Although traditional practitioners do not have a clear picture to explain why this type of pretreatment is done, they believe it reduces toxicity associated with plumbagin. Here, we report a preliminary attempt to give a scientific basis for the pretreatment by using UV/Vis spectrophotometric and chromatographic methods.

Shodhana process resulted a deep maroon colour extract substantiating plumbagin gives a red color in alkaline pH. After recrystallization using hexane, plumbagin was obtained as orange needles and melting point of 76-77 °C was in accordance with literature. In GC/MS studies, the gas chromatogram showed a single peak and corresponding mass spectrum a molecular ion at m/z 188. The IR and UV results were also in accordance with what is published and confirmed the purity of isolated plumbagin. In the study of pretreatment, three hexane extracts (E_1 – hexane extract of fresh roots upon subjecting to pretreatment, E_2 – hexane extract of used roots after pretreatment, and E_3 – hexane extract of fresh roots without subjecting to pretreatment) were subjected to TLC against isolated plumbagin which was used as the working standard using 9:1, benzene: hexane solvent system. According to the results, E_3 showed a very intense spot (S_1) with R_f 0.63 that correspond to the plumbagin working standard and six other spots of which two were very intense (S_2 and S_3) while others were of low intensity. In the case of E_1 , the S_1 spot was observed with low intensity, S_2 spot was not observed at all while the other spots were in low intensities of which one was an extra spot (S_4). When considering E_2 , the S_1 , S_2 and S_3 spots were observed intensively (not as intense as in E_3)

while the others in low intensities. All these collectively substantiate the fact that although the pretreatment process does not cause much change in the phytochemical composition of the roots, it causes a reduction in the amount of plumbagin along with the other compounds but not completely. By employing optimized conditions and using the calibration curve, which was developed, by using isolated plumbagin as the working standard, the fresh root sample quantified 8.7 ± 0.1 mg/g of plumbagin, which was lowered by 19.4% upon subjecting to pretreatment. Likewise commercial sample quantified 0.55 ± 0.05 mg/g of plumbagin. There is a large difference in amount of plumbagin between fresh and commercial root samples. This may be due to plumbagin being eliminated during the drying process that commercial samples under go before being marketed. According to anecdotal evidences and published reports, the pretreatment is done to reduce toxicity associated with plumbagin. However, ayurveda formulations are incorporated with air-dried roots (commercial samples) not with fresh roots. If pretreatment is done only to reduce toxicity associated with plumbagin, drying process may be sufficient to reduce plumbagin and it may be possible to exclude the pretreatment in ayurvedic preparations. However, to better understand the changes in phytochemical composition of roots of *P.indica* during drying, a long-term qualitative and quantitative study is under way.

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Decarboxylation of Waste Coconut Oil for the Production of Green Diesel

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Green diesel has emerged as an environmentally and economically friendly solution to the energy crisis. Green diesel can be produced either by hydrogenation or decarboxylation/decarbonylation. This project is involved in producing hydrocarbons from waste coconut oil by decarboxylation. Waste coconut oil was filtered with anhydrous Sodium Sulphate and heated. The waste coconut oil was hydrolyzed using ethanolic KOH to yield free fatty acids. Hydrolysis was carried out at 60 °C for 2 hours. After alkaline hydrolysis, the mixture was acidified using glacial acetic acid. To determine whether hydrolysis has taken place acid values of oil before and after hydrolysis were compared. The decarboxylation process was carried out by a special apparatus designed by the authors.

Decarboxylation process was carried out at 200 °C and the fractions of product were collected in the collecting vessel. The mixture was then distilled and two fractions were collected at 60-80 °C and 80-110°C. Distilled fractions and the remaining residue were extracted into petroleum ether, water layer separated and analyzed by GC-MS at the University of Colombo. The distillation fraction at 60 °C- 80 °C showed the presence of hydrocarbons. These hydrocarbons are nonane, decane, undecane, dodecane, tridecane and are in the petro diesel range. This process can be improved by applying high pressure and temperature. Work is being carried out using a high pressure reactor and Pd/C as a catalyst.

Anti-diabetic compounds in *Syzygium cumini* ready to serve herbal drink

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Herbal beverages with desirable sensory attributes are an ideal way to offer consumers with phytochemicals having specific health promoting functionalities. *Syzygium cumini* bark decoction is used in treating diabetes mellitus in Ayurvedha medicine¹. Based on the findings of earlier research work of the authors in relation to antidiabetic properties of *S. cumini* decoction, such as antiglycation and antioxidant activities and high total phenolic content, a ready to serve (RTS) herbal drink was developed. This work describes the chemistry of the *S. cumini* decoction and the RTS herbal drink developed. The decoction was prepared according to the traditional method used to prepare decoctions in Ayurvedha medicine using commercial samples.

Activity guided fractionation of the decoction of the *S. cumini* was carried out by sequential extraction of organic solvents with different polarities. Ethyl acetate and aqueous fractions were analyzed using different chromatographic methods to determine the active compounds. Phenolic compounds of the ethyl acetate extract of the decoction were determined using Thin

Layer Chromatography (TLC) method and by comparing R_f values with authentic compounds. High Performance Liquid Chromatography (HPLC) analysis was performed for the identification and confirmation of the compounds in the decoction and the RTS herbal drink.

Gallic acid ($R_f = 1.7\text{min.}$) and ellagic acid ($R_f = 3.65\text{min.}$) were separated by HPLC, on a C18 column using 1% acetic acid and acetonitrile (80:20 v/v). An UV-VIS library of pure compounds were created using Millennium chromatographic manager package by injecting the pure compounds to the HPLC under the above chromatographic conditions. The LC UV-VIS spectra of the two compounds were identical with the corresponding spectra of the library. Gallic acid and umbelliferone were determined as the active compounds in the decoction by TLC method and were confirmed by applying the co-chromatography with authentic compounds. Gallic acid and ellagic acid were determined through the HPLC analysis as the active ingredients in the decoction and in the RTS herbal drink and the presence of these compounds were confirmed

by spiking the samples with authentic compounds.

The antidiabetic activities of gallic acid², ellagic acid³ and umbelliferone⁴ have already been proven by several studies. The findings of the present investigation confirmed the presence of these compounds in *S. cumini* decoction and also the RTS drink prepared with the decoction. Therefore, these findings support in proving the antidiabetic properties and thus the efficacy of using *S. cumini* in the treatment of diabetes mellitus.

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Key words: *Syzygium cumini* decoction, RTS herbal drink, gallic acid, ellagic acid, phenolic compounds, diabetes mellitus

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Technical Sessions : A - 17

Isolation and Characterization of probiotic “*Pediococcus acidilactici*” from Sri Lankan finger millet variety (*Elucine coracana*)

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Finger millet is cultivated and consumed in Sri Lanka and has many health benefits. It is a good prebiotic source, which provides the required conditions for the growth of probiotic bacteria. *Pediococcus acidilactici*, a probiotic bacterium that produces anti *Helicobacter pylori* bacteriocin is currently used as an alternative therapy for peptic ulcers and gastritis caused by *H. pylori*. The objective of this study is to isolate and characterize lactic acid bacteria from Sri Lankan finger millet (*Elucine coracana*) “Oshadha” cultivar. Seeds were collected from the germplasm of Seed and Planting Material Centre at Pelwehera and milled and sieved, flour was fermented for 19 h at 30 °C. Isolation of lactic acid bacteria was carried out by serial dilution followed by spread plate technique on de Man Rogosa and Sharpe Agar (MRS agar). The isolate was further characterized for phenotypic parameters by Gram and endospore staining, motility and biochemical parameters which include; indole, methyl red, vogues prosker, citrate, gelatin liquefaction, H₂S production, starch hydrolysis, urease and catalase tests. Acid, bile, sodium chloride and temperature tolerance and sugar fermentation patterns for Maltose, Lactose, Glucose,

Sorbitol, Arabinose, Mannitol and Dextrose of the isolate were evaluated. Bacterial DNA was extracted, purified and 16S rRNA sequencing was carried out. The result revealed that the isolate was a Gram positive, non sporeforming, non motile cocci bacteria which fermented all sugars, was positive for methyl red and negative for all other biochemical tests performed. Isolate tolerated high acidic conditions (pH 3, 4) and bile concentrations of 0.3%, 0.6%, 0.8%, temperatures of 30 °C, 37 °C, 42 °C and tolerate sodium chloride concentrations of 5.5%, 6.5% 7.5%. 16S rRNA sequencing analysis of the amplified gene identified the isolate as the lactic acid bacteria, *P.acidilactici*. This is the first report of the isolation and characterization of *P.acidilactici* from Sri Lankan finger millet. Further evaluation of bacteriocin production and the safety aspects of the isolate will result in the establishment of a starter culture for the development of finger millet based probiotic products.

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Food and Nutritional Security through Novel Cereal and fruit based prebiotics”) is gratefully acknowledged.

Technical Sessions : A - 18

***In vitro* starch digestibility and resistant starch content of selected banana varieties (*Musa species*) from Sri Lanka**

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Resistant starch plays an important role in weight management, intestinal or colonic health as well as helps to lower blood glucose levels and improve insulin sensitivity. Prebiotic effects of resistant starch in the gut and its impact on gut health is also well documented. Banana is commonly cultivated and consumed by almost all Sri Lankans in their normal diet. Therefore, the main objective of this study is to quantify the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents of nine selected banana varieties, namely, *Seeni*, *Seeni parakum*, *Kandula*, *Anamalu*, *Kolikottu*, *Nadee ambul*, *Nethrampalam*, *Rathkesel* and *Embon*.

The banana samples were collected from Research Station, Department of Agriculture, Giradurukotte, Regional Agriculture Research and Development Center, Angunakolapelessa and Agriculture Research Center, Thelichawila. Moisture content of all banana varieties was determined following AOAC method². Rapidly digestible starch (RDS), SDS and RS contents of banana flour were measured by modified *Englyst* method for *in vitro* starch digestibility¹. This method involves the enzymatic hydrolysis of starch with pancreatin, amyloglucosidase and invertase under controlled conditions and colorimetric quantification of released glucose by using Glucose oxidase peroxidase (GOPOD) test kit.

The moisture content of these selected banana varieties varies between 60.28% and 74.94% and the moisture content of banana flour varied between 11.50% and 13.21%. Rapidly digestible starch content of *Seeni*, *Seeni parakum*, *Kandula*, *Anamalu*, *Kolikottu*,

Nadee ambul, *Nethrampalam*, *Rathkesel* and *Embon* were respectively, $3.54 \pm 0.30\%$, $4.33 \pm 1.17\%$, $8.92 \pm 1.04\%$, $6.66 \pm 1.84\%$, $2.00 \pm 0.73\%$, $3.00 \pm 1.04\%$, $3.88 \pm 0.57\%$, $10.47 \pm 0.03\%$ and $3.56 \pm 1.05\%$ while SDS contents were at $7.44 \pm 0.83\%$, $6.63 \pm 0.89\%$, $5.67 \pm 0.30\%$, $2.56 \pm 0.61\%$, $4.22 \pm 1.46\%$, $4.56 \pm 1.01\%$, $1.69 \pm 0.58\%$, $4.28 \pm 0.38\%$ and $0.84 \pm 0.36\%$ correspondingly. Rapidly digestible starch content was high in *Rathkesel* ($10.47 \pm 0.03\%$) and relatively low in *Kolikottu* ($2.00 \pm 0.73\%$). There is a significant difference ($p < 0.005$) in the RDS content of studied banana varieties. High amount of SDS has reported for *Seeni* ($7.44 \pm 0.83\%$) while lowest amount was recorded for *Embon* ($0.84 \pm 0.36\%$). A significant difference ($p < 0.05$) was observed in SDS content of these banana varieties. *Nethrampalam* is reported as rich in resistant starch content at $71.99 \pm 0.39\%$ and followed by other banana varieties in the order of *Anamalu* ($64.46 \pm 6.48\%$), *Nadee ambul* ($64.08 \pm 2.06\%$), *Kolikottu* ($60.00 \pm 0.53\%$), *Embon* ($59.57 \pm 1.90\%$), *Kandula* ($56.28 \pm 4.39\%$), *Seeni* ($52.59 \pm 1.46\%$), *Seeni parakum* ($51.89 \pm 2.46\%$) and *Rathkesel* ($43.93 \pm 0.70\%$). Resistant starch contents of these banana varieties are significantly different ($p < 0.05$). This study indicated that local banana varieties have average of $4.19 \pm 0.84\%$ of RDS, $5.57 \pm 1.03\%$ of SDS and $58.31 \pm 6.44\%$ of RS.

As these banana varieties are affluent in resistant starch content, indicating it as a prebiotic source. Further analysis is required and will be carried out to investigate the prebiotic properties of these selected varieties.

Professor M U S Sultanbawa Award for Research in Chemistry

This award is being made from the Professor M U S Sultanbawa Felicitation fund, which was established on the occasion of his 75th birthday to recognize the unique, distinguished and significant contribution made to the cause of Science, Chemistry, Education and Research in Sri Lanka by Vidya Jyothi Professor Mohamed Uvais Siddeek Sultanbawa.

This award is made annually for the Best Research paper presented at the Annual Sessions for the work carried out and completed in Sri Lanka.

Professor M U S Sultanbawa Award - 2014



Mr. S C Dilanka Fernando

Mr. S C Dilanka Fernando who is a Graduate Chemist has been awarded the “Sultanbawa Award - 2014” in recognition of his research on “***In-vitro* radical scavenging properties, anti-inflammatory and α -amylase inhibitory activities of *Eriocaulon quinquangulare* aqueous extract**”. This research has been supervised by Professor S S S B D Preethi Soysa at Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Colombo.

Mr. S C Dilanka Fernando obtained Graduateship in Chemistry in 2010 with a First Class Honours. He obtained his Master's degree in Biochemistry & Molecular Biology from the Faculty of Medicine, University of Colombo. His research work was focused on the area of pharmacognosy and bio-activity studies of various plant based medicaments under the supervision of Prof. S S S B D Preethi Soysa. He is a Corporate Member of the Institute of Chemistry and an Associate Member of the Royal Society of Chemistry, UK. Currently, he works as a Senior Teaching Assistant at College of Chemical Sciences, Institute of Chemistry Ceylon.

***In-vitro* radical scavenging properties, anti-inflammatory and α -amylase inhibitory activities of *Eriocaulon quinquangulare* aqueous extract**

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1. Introduction

During hepatic drug biotransformation, free radicals are continuously generated. Free radicals are highly reactive, unstable molecules that react rapidly with adjacent molecules via a variety of reactions including: hydrogen abstraction, electron capturing and electron sharing (McCord, 2000) leading to lipid peroxidation, protein oxidation, DNA strand breaks, and modulation of gene expression. Experimental evidences show that these free radicals are involved in liver diseases (Hikino & Kiso, 1988) and also lead to atherosclerosis, cancer, stroke, asthma, arthritis and other age related diseases (Liao and Yin, 2000).

Plants as natural source of antioxidants have the potential to scavenge free radicals as well as to inhibit their generation (Ravishankar *et al.*, 2013). It is a well established fact that plants having antioxidant properties also exert hepatoprotective activity (Feher *et al.*, 1986). At present the toxic effects of synthetic antioxidants have been reported, hence the interest for searching of natural antioxidants of plant origin has increased greatly during recent times (Jayaprakash and Rao, 2000). Medicinal practices using plant derived

drugs have shown that these particular drugs are relatively non-toxic, safe and even free from serious side effects (Momin A., 1987).

Eriocaulon quinquangulare (Family: Eriocaulaceae) locally known as “Heen kokmota” is a slender annual tuft. This monocotyledonous plant is distributed in lowlands in Sri Lanka (Dassanayake and Clayton, 1997). The total plant of *Eriocaulon quinquangulare* prepared as decoction is used to treat patients suffering from liver disorders, jaundice and splenomegaly in Sri Lanka (Ediriweera, 2007).

Due to lack of scientific investigations done so far, this study was designed to investigate the phytochemical constituents, *in-vitro* radical scavenging properties and anti-inflammatory properties of afore mentioned plant extract. Additionally, this plant extract was also assayed for α -amylase inhibitory activity.

2. Materials and Methods

2.1. Chemicals and equipment

The chemicals gallic acid, Folin ciocalteu reagent, trichloroacetic acid, horse radish peroxidase and

ethylenediamine tetra acetic acid (EDTA) were purchased from Sigma Chemicals Co. (P.O. Box 14508, St. Louis, MO 63178 USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical, (-)-epigallocatechin gallate, aluminium chloride and sulfanilamide were purchased by Fluka (Fluka chemie GmbH, CH-9471 Buchs). L-ascorbic acid, hydrogen peroxide, N-(1-Naphthyl)-ethylene diamine dihydrochloride and ethanol were purchased from BDH Chemicals (BDH Chemicals Ltd, Poole, England). Sodium nitroprusside was purchased from Qualigens (A division of GlaxoSmith Kline Pharmaceuticals Ltd). Ferric chloride, potassium ferricyanide and sodium nitrite were purchased from Riedel De Haen Ag, Wunstorfer Strasse 40, SEELZE1, D3016, Germany.

Decoctions were freeze dried using LFT 600EC freeze dryer. SHIMADZU UV 1601 UV Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used to read the absorbance. The samples were centrifuged using Biofuge pico D-37520 (Heraeus instruments) centrifuge.

2.2. Plant material

The whole plant of *Eriocaulon quinquangulare* (Kokmota) was collected from Kalutara District. This plant material was identified and confirmed by Department of Botany, Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Sri Lanka.

2.3. Preparation of the decoctions

Decoctions from total plant of *Eriocaulon quinquangulare* were prepared according to a procedure followed by Ayurvedic practitioners of Sri Lanka. Six to nine plants pooled together was used to prepare the decoction. The plant material was washed separately with tap water followed by distilled water and de-ionized water and dried to achieve a constant weight. A weight of 30g of plant material was cut into small pieces, ground to a fine powder using a clean kitchen blender and was boiled with 800ml of deionized water until total volume reduced to 100ml (1/8th of the original volume) in an opened glass beaker. The decoction was sonicated, filtered and the filtrate was centrifuged at 2000 rpm for 10min. The supernatant was freeze dried. The freeze dried sample were weighed, and stored at -20 °C in sterile tubes until further use. The yield was calculated as a percentage of dry weight used.

2.4. Determination of Total Phenolic Content

Total phenolic content of the decoction of *Eriocaulon quinquangulare* was determined by Folin ciocalteu method (Makkar *et al.*, 1993). Calibration curve was constructed using gallic acid standards (6-30 µg/ml) and the total phenolic content of above

decoctions were expressed as w/w% gallic acid equivalents.

2.5. Determination of Flavonoid Content

The flavonoid content was measured by the aluminium chloride colorimetric assay (Zhishen *et al.*, 1999). Calibration curve was plotted using (-)-epigallocatechin gallate (EGCG) standards (0.3-1.0 mg/ml) and flavonoid content was expressed as w/w% EGCG equivalents.

2.6. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free radical Scavenging Activity

Free radical scavenging ability of the decoction prepared was assessed by DPPH radical scavenging method described by Blois, (1958) with slight modification. Different concentrations of *Eriocaulon* (10-250 µg/ml) were prepared in deionized water. DPPH reagent prepared in absolute ethanol (100 µM, 750 µl) was added to test sample (250 µl) and the mixture was allowed to stand for 30 minutes in the dark. The scavenging activity by each concentration was quantified by measuring the decolourization of the resulting solutions at 517 nm. Since the water extract of *Eriocaulon* had a colour of brown which interferes with the colour of DPPH, blank was prepared totally with deionized water and separate samples for each concentration of *Eriocaulon* (250 µl) mixed with deionized water (750 µl) was used to measure interference by the background and later on this reading was subtracted from the original reading. The control was prepared by mixing deionized water (250 µl) with DPPH (750 µl). L-Ascorbic acid (concentration series of 2-15 µg/ml) was prepared as standard reference antioxidant. Results were expressed as percentage inhibition (% I) calculated using Equation 1:

$$\% \text{ Inhibition} = \frac{\text{Abs.of control} - \text{Abs.of sample}}{\text{Abs.of control}} \times 100\% \quad (\text{Equation 1})$$

The effective concentration needed to scavenge 50% of the DPPH free radical (EC₅₀) was calculated by regression analysis of the dose response curve plotted between percentage inhibition versus concentration of the test samples and the standard.

2.7. Hydrogen peroxide (H₂O₂) scavenging activity

Hydrogen peroxide scavenging activity of the decoctions prepared was determined according to Fernando and Soysa, (2015). The developed method utilizes the reaction where hydrogen peroxide rapidly reacts with phenol and 4-aminoantipyrine in the presence of horseradish peroxidase to produce a quinoneimine chromogen which is pink coloured that

can be measured at 504 nm. H_2O_2 scavengers will eventually result in decreased production of this particular chromophore. L-Ascorbic acid (concentration series of 12-40 $\mu\text{g/ml}$) was used as reference standard antioxidant. The percentage inhibition of hydrogen peroxide was calculated according to Equation 1. The effective concentration required to scavenge by 50% of hydrogen peroxide in the system (EC_{50} value) was calculated from the dose response curve plotted between % inhibition versus concentration of test samples and the standard.

2.8. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of the decoction prepared was measured based on Griess - Ilosvay reaction (Garret, 1964) with slight modification. Nitric oxide generated spontaneously from sodium nitroprusside (SNP) in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess reagent. The diazonium salt formed by the reaction of nitrite ions with sulfanilamide couples with N-(1-Naphthyl)-ethylene diamine dihydrochloride to form an azo-dye which can be measured spectroscopically at 540 nm. Scavengers of nitric oxide compete with oxygen which leads to reduced production of nitrite ions and ultimately reduced production of the azo-dye. L-ascorbic acid (concentration series of 200-2000 $\mu\text{g/ml}$) was used as reference standard antioxidant. The percentage scavenging was calculated using Equation 1. The effective concentration required to scavenge 50% of the nitric oxide free radicals generated in the system (EC_{50} value) was calculated from the dose response curves of test samples and the standard.

2.9. Estimation of membrane stabilizing activity using hypotonic solution induced human erythrocyte hemolytic assay

The membrane stabilization activity of the decoction prepared was determined according to the procedure described by Sadique *et al.*, (1989) with slight modifications. Fresh whole human blood (5 ml) was collected and transferred to centrifuge tubes. The tubes were centrifuged at 2500 rpm for 5 min and the supernatant was removed. The cell suspension was washed 3-4 times with isotonic buffer (154 mM sodium chloride in 10 mM phosphate buffer, pH 7.4) until the supernatant appeared clear. The volume of blood was measured and reconstituted as 40% v/v suspension with isotonic buffer.

Human erythrocyte suspension (40% v/v, 50 μl) was mixed with hypotonic buffer (50 mM sodium chloride in 10 mM phosphate buffer, pH 7.4, 1.0 ml) and plant extract prepared in isotonic buffer (100 μl).

The samples were incubated for 20 minutes at room temperature followed by centrifugation at 5000 rpm for 5 minutes. The absorbance of the supernatant was measured at 540 nm. The supernatant obtained after incubation of erythrocyte suspension with isotonic buffer, served as the reagent blank. The supernatant obtained after incubation of erythrocyte suspension with hypotonic buffer, served as the control. Appropriate samples were constructed to nullify background interferences caused by the plant extract. Sodium salicylate was used as standard anti-inflammatory drug. Percentage inhibition of hemolysis (% I) caused by the plant extract and sodium salicylate was measured according to Equation 1. EC_{50} values were estimated using regression analysis of the dose response curves plotted between % inhibition of hemolysis versus concentration.

2.10. In-vitro α -amylase inhibitory activity

The α -amylase inhibitory activity was assessed by the standard method described by Dong *et al.*, (2012) with slight modifications. Test sample (100 μl) was premixed with α -amylase solution (2.0 U/ml, 100 μl) prepared in buffer (0.02 M phosphate buffer with 6 mM sodium chloride, pH 6.9) and incubated at 25 °C for 10 min. After pre-incubation, starch solution (1 % w/v, 100 μl) prepared in same buffer was added to the mixtures to start the reaction. The reaction was allowed to occur at 25 °C for 5 min and terminated by addition of 100 μl of the DNSA reagent (1% 3,5-dinitrosalicylic acid and 30 % sodium potassium tartrate in 0.4 M NaOH). The samples were incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted up to 1.0 ml with distilled water and absorbance was measured at 540 nm against reagent blank where plant extract and enzyme was replaced with the buffer. Control samples retain 100% enzyme activity and were conducted similarly by replacing plant extract with buffer. Since the plant extract exhibited a colour which interferes with the red colour of reduced dinitrosalicylic acid formed in the reaction, appropriate samples were constructed for background subtraction. Tannic acid which is a known potent amylase inhibitor was used as the positive control. The α -amylase inhibitory activity was expressed as % inhibition which was calculated using Equation 1. EC_{50} values were estimated using regression analysis of the dose response curves plotted between % inhibition versus concentration.

Statistical Analysis

A minimum of three independent experiments were carried out unless otherwise specified. Regression analysis and statistical analysis were carried out using

Microsoft Excel. Calibration curves of the standards were considered as linear if $R^2 > 0.99$. EC_{50} values were calculated from either linear or logarithmic dose response curves where $R^2 > 0.90$.

Results and discussion:

The total plant of *Eriocaulon quinquangulare* (Family: Eriocaulaceae) prepared as decoctions/herbal porridges are prescribed by Sri Lankan traditional medicinal practitioners for the treatment of various liver diseases (Ediriweera, 2007). Its' anti-inflammatory and α -amylase inhibitory properties are not yet confirmed scientifically. The current study is therefore focused to determine these activities as well as *in-vitro* antioxidant properties of lyophilized decoctions prepared from the above plant material.

Eriocaulon quinquangulare total plant gave an extraction yield of 4.33 as a percentage of dry weight of sample used. Phenolic compounds act as primary antioxidants or free radical scavengers due to their hydroxyl groups possessing scavenging ability (Dhalwal *et al.*, 2008). The Folin- Ceocalteu reaction was used to determine total phenolic content of the decoction prepared. *E. quinquangulare* exhibited total phenolic content value of 10.32 ± 1.63 w/w% gallic acid equivalents (as calculated using the standard curve for gallic acid ie; Figure 1) indicating *E. quinquangulare* contains a higher content of phenolic compounds.

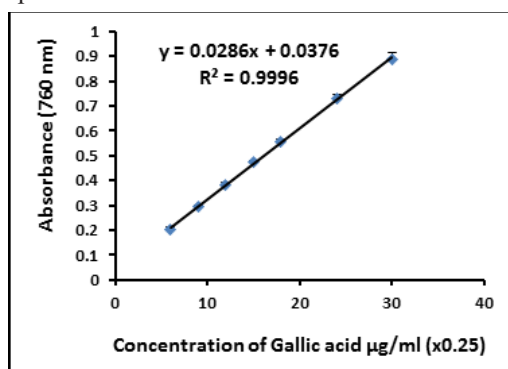


Figure 1: The calibration curve for gallic acid which was used to determine the total phenolic content of the decoction. The values for absorbance are presented as mean + SD of six independent experiments.

Studies conducted with the water extract of *Eriocaulon sexangulare* L. found in Taiwan has yielded a total phenolic content of 88.62 ± 0.91 µg Catechin equivalents/mg (Yu-Ling *et al.*, 2012) compared to *E. quinquangulare* in our study where the total phenolic content was expressed in Gallic acid equivalents. Flavonoids are considered as potential antioxidants exerting their antioxidant activity by the mechanisms of radical scavenging and metal ion chelation to inhibit

lipid peroxidation (Oboh *et al.*, 2007). Aluminium chloride colorimetric assay for flavonoids yielded total flavonoid content of 45.55 ± 3.77 w/w% (-)-Epigallocatechin gallate (EGCG) equivalents for *E. quinquangulare* (as calculated using the standard curve for EGCG ie; Figure 2) indicating that *E. quinquangulare* has a very high total flavonoid content. Studies conducted with water extract of *Eriocaulon sexangulare* has yielded a total flavonoid content of 9.57 ± 0.25 µg Rutin equivalents/mg (Yu-Ling *et al.*, 2012) compared to *E. quinquangulare* in the present study where the total flavonoid content was expressed in EGCG equivalents.

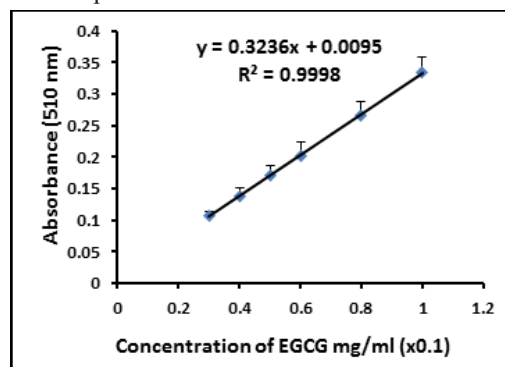


Figure 2: The calibration curve constructed using (-)-epigallocatechin gallate (EGCG) standards to determine the total flavonoid content of the decoctions. The values for absorbance are presented as mean + SD of nine independent experiments.

Many researchers have stressed the need to perform more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action in order to estimate total antioxidant potential of plant extracts (Frankel and Meyer, 2000). In accordance with this prospect, total antioxidant activities of the decoctions prepared were evaluated using DPPH and nitric oxide radical scavenging assays as well as H_2O_2 scavenging assay. Antioxidant potential of the plant extracts against the radical systems was determined by calculating the EC_{50} value (half maximal effective concentration) from the corresponding dose response curves via linear or logarithmic regression analyses. The EC_{50} values obtained in this manner make it convenient in comparative studies with other plant extracts assessed for similar antioxidant activity. Lesser the EC_{50} value, higher will be the antioxidant potential of a particular plant extract.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical was used to determine hydrogen donating ability of the plant extracts used. Being a stable free radical DPPH will not undergo dimerization a quality found mostly with other radicals as well. The free

radical which is centred on Nitrogen atom due to being delocalized within the aromatic system gives a characteristic purple colour which is measured around 517 nm. In the presence of hydrogen donors ie, free radical scavengers, DPPH reacting with these hydrogen atoms yield a stable product 1,1-Diphenyl-2-picrylhydrazine resulting in a colour change from purple to yellow (Blois, 1958; Molyneux, 2004). In the current study *E. quinquangulare* exhibited EC_{50} value of $37.18 \pm 1.69 \mu\text{g/ml}$ and L-Ascorbic acid exhibited EC_{50} value of $3.30 \pm 0.27 \mu\text{g/ml}$. The results indicate that L-Ascorbic acid has a hydrogen donating ability greater than that of *E. quinquangulare*. However *E. quinquangulare* extract in the current research demonstrated greater hydrogen donating ability than *Eriocaulon sexangulare* L. extract as implied by DPPH radical scavenging assay ($EC_{50} > 2000 \mu\text{g/ml}$) (Yu-Ling *et al.*, 2012).

Although hydrogen peroxide itself is not very reactive, it can generate the highly reactive hydroxyl radical ($HO^\bullet$) through the Fenton reaction (Halliwell, 1991). Therefore, scavenging of hydrogen peroxide is also considered as an important feature of antioxidants (Duh *et al.*, 1999). Accepting electrons in the presence of electron donors, hydrogen peroxide is decomposed into water. Hydrogen peroxide scavenging activity especially of phenolic compounds is assigned to such electron-donating ability (Wettasinghe and Shahidi, 2000). The amount of pink coloured chromogen formed in the reaction between hydrogen peroxide, phenol and 4-aminoantipyrine (catalyzed by horseradish peroxidase) decreased in a dose dependant manner of the decoctions due to their scavenging ability of hydrogen peroxide molecules. Present study shows that, EC_{50} values for hydrogen peroxide scavenging activity are 381.98 ± 1.83 and $10.01 \pm 0.14 \mu\text{g/ml}$ for *Eriocaulon* and L-Ascorbic acid respectively implying that *E. quinquangulare* has a lesser scavenging ability of hydrogen peroxide than L-Ascorbic acid.

Nitric oxide (NO), is an important chemical mediator produced by endothelial cells, macrophages, neurons and it regulates various physiological processes like vasodilation, neurotransmission, synaptic plasticity and memory in the central nervous system (Bredt and Snyder, 1994). During conditions like infections and inflammation, formation of NO is elevated and in the aerobic environment NO will react with oxygen to produce intermediates such as NO_2 , N_2O_4 , N_3O_4 , the stable products nitrate and nitrite (Marcocci *et al.*, 1994) and peroxynitrite anion ($ONOO^-$) when reacted with superoxide (Wink *et al.*, 1991). Although NO doesn't interact with biological macromolecules like proteins and nucleic acids

directly, the aerobic products formed from NO as mentioned above especially peroxynitrite anion ($ONOO^-$) are strong oxidants (Malinski, 2007) and will give rise to adverse effects such as DNA fragmentation, cell damage and neuronal cell death (Dawson *et al.*, 1992). In the experiment done, nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite which is estimated using Griess reagent. The EC_{50} values obtained were 31.85 ± 2.22 and $276.3 \pm 25.8 \mu\text{g/ml}$ for *E. quinquangulare* and L-ascorbic acid respectively. These results suggest that *E. quinquangulare* is a more potent NO scavenger than L-Ascorbic acid. The concentration of the decoction required for scavenging 50% of the DPPH, H_2O_2 and nitric oxide (EC_{50}) were determined using the dose response curves obtained (Figures 3-5).

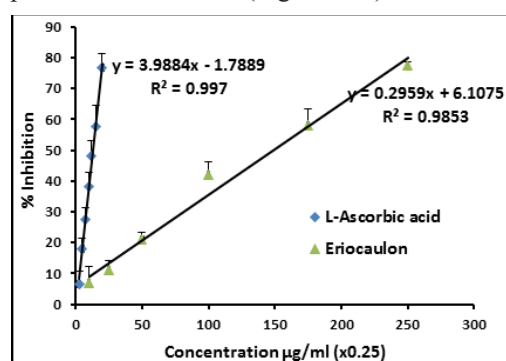


Figure 3: The dose response curves for percentage scavenging of DPPH by *Eriocaulon* decoction in comparison with L-ascorbic acid. The results are presented as mean + SD for L-ascorbic acid (n=9) and *Eriocaulon* (n=3).

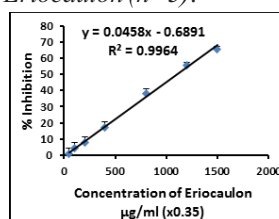


Figure 4(a)

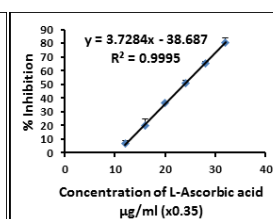


Figure 4(b)

Figure 4: The dose response curves for percentage scavenging of H_2O_2 by *Eriocaulon* decoction in comparison with L-ascorbic acid. The results are presented as mean + SD for three independent experiments.

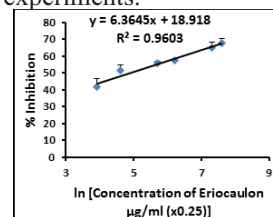


Figure 5(a)

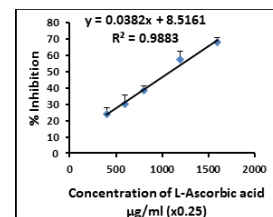
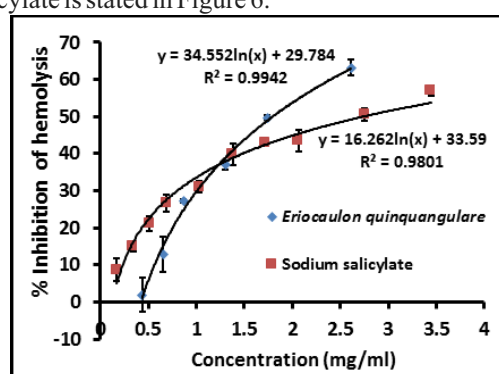


Figure 5(b)

Figures 5: The dose response curves for percentage inhibition of nitric oxide radicals by *E.*

quinguangulare decoction [Figure 5(a)] and L-ascorbic acid [(Figure 5(b)]. The results are presented as mean $\pm$ SD of three independent experiments.

The erythrocyte membrane is analogous to the lysosomal membrane and a plant extract well stabilizing the lysosomal membrane implies that it limits the release of lysosomal enzymes from lysosomes in activated neutrophils into the surrounding tissue. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Sadique *et al.*, 1989). The EC_{50} value of 1.794 ± 0.045 mg/ml elicited by the plant extract for human red blood cell (HRBC) membrane stabilization assay suggests that it can prevent hypotonic solution induced HRBC membrane rupture appreciably when compared to the standard anti-inflammatory drug, sodium salicylate ($EC_{50} = 2.548 \pm 0.083$ mg/ml) in a dose dependent manner. The dose response curves for the plant extract and sodium salicylate is stated in Figure 6.



Figures 6: The dose response curves for percentage inhibition of hemolysis by *E. quinguangulare* decoction and standard anti-inflammatory agent sodium salicylate. The results are presented as mean $\pm$ SD of three independent experiments.

One anti-diabetic therapeutic approach to reduce postprandial glucose level in blood is by the inhibition of pancreatic α -amylase enzyme (Dong *et al.*, 2012). *E. quinguangulare* had a capability of inhibiting pancreatic α -amylase with an EC_{50} value of 1.383 ± 0.066 mg/ml with respect to potent amylase inhibitor, tannic acid ($EC_{50} = 0.018 \pm 0.003$ mg/ml). This indicates that the plant extract possess an ability to reduce postprandial hyperglycemia via inhibition of pancreatic α -amylase. The corresponding dose response curves for the plant extract and sodium salicylate is stated in Figures 7(a) and 7(b) respectively. The summary of the results obtained for all experiments is indicated in Table 1.

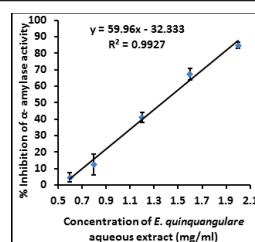


Figure 7(a)

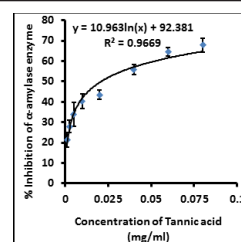


Figure 7(b)

Figures 7: The dose response curves for percentage inhibition of α -amylase enzyme activity by *E. quinguangulare* decoction [Figure 7(a)] and tannic acid [Figure 7(b)]. The results are presented as mean $\pm$ SD of three independent experiments.

Table 1: Summary of the results obtained for different experiments.

Name of the experiment	<i>Eriocaulon quinguangulare</i>	
Phenolic content (w/w% Gallic acid equivalents)	10.32 $\pm$ 1.63	
Flavonoid content (w/w % (-)-Epigallocatechin gallate equivalents)	45.55 $\pm$ 3.77	
Name of the experiment	EC_{50} for <i>Eriocaulon quinguangulare</i>	EC_{50} for Positive control
DPPH radical scavenging activity	37.18 $\pm$ 1.69 μ g/ml	L-Ascorbic acid, 3.30 $\pm$ 0.27 μ g/ml
Nitric oxide scavenging activity	31.85 $\pm$ 2.22 μ g/ml	L-Ascorbic acid, 276.3 $\pm$ 25.8 μ g/ml
H ₂ O ₂ scavenging activity	381.98 $\pm$ 1.83 μ g/ml	Gallic acid, 7.82 $\pm$ 0.19 μ g/ml
Membrane stabilization effect	1.794 $\pm$ 0.045 mg/ml	Sodium salicylate, 2.548 $\pm$ 0.083 mg/ml
α -amylase inhibitory activity	1.383 $\pm$ 0.066 mg/ml	Tannic acid, 0.018 $\pm$ 0.003 mg/ml

Conclusion:

Our findings suggest that the decoction prepared from *E. quinguangulare* has the potential to act as a strong radical scavenger, anti-inflammatory agent and α -amylase inhibitor. These properties may be attributed to considerable high amounts of phenolics and flavonoids present, hence justifying its folkloric utilization in the treatment of health complications including inflammatory processes. These raw plant materials are suitable for the application in nutritional/pharmaceutical fields and in the prevention of free radical mediated diseases. The quantification of the plant's individual phytoconstituents as well as pharmacological profile based on *in-vivo* studies and clinical trials should be further investigated.

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Guest Articles

Determination of Residue Estrogens in Environmental Matrices

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Estrogen hormones excreted to the environment by humans and wildlife are capable of making deleterious impact on aquatic organisms even at extremely low concentrations.¹ They are known to be the most potent endocrine disruptors in the environment as a result of their high affinity to estrogen receptors.²⁻⁵ For example, vitellogenin production in rainbow trout by 17 β -estradiol (β E2) has been reported at low parts per trillion environmental concentrations.^{2,6}

In general, humans use significant amounts of estrogens as medicine,⁷⁻⁸ and also the bodily organs secrete natural hormones. After excreted in urine and feces, these hormones are eventually discharged into municipal wastewaters.⁹⁻¹¹ Therefore, hazardous amounts of estrogens can be accumulated to wastewaters in urban areas with high population densities. Hence in such areas, there is a possible risk of accumulating hazardous amounts of estrogens to wastewater influents, which are expected to be removed by the treatment process of the wastewater treatment plant (WWTP). Thus, WWTPs are a potential pathway of environmental endocrine disruptors since any estrogens not removed during treatment release to the environment.

Concentrated animal feeding operations (CAFOs) have been identified as the other potentially important pathway for the release of estrogens into the environment because of high fecal and urine excretion rates.¹²⁻¹³ Release of estrogens from swine and cattle operations and their environmental impact has become an emerging concern as CAFO production is used worldwide. Lagoon wastewater is typically discharged

onto fields to meet crop nutrient requirements. This increases the potential of estrogens entering the surrounding water systems from surface runoff during storm or snowmelt events.¹²

Analysis of estrogen residues in the aquatic environment is a challenging task mainly due to the complexity of the matrix and the demand for extremely low detection limits. Environmental aqueous samples such as municipal wastewater, lagoon water, ground water, surface water, etc. usually contain numerous compounds that can interfere in the analysis of the target analytes. Also, since these compounds have been reported to cause adverse effects in living organisms at low parts per trillion detection limits, the detection methods should be capable of determining such low environmental concentrations. A typical analytical method consists of a sample pre-concentration step, sample cleanup step(s), and a powerful detection method in order to address these challenges.¹⁴⁻²²

Sample Preparation

Sample Collection and Storage: Upon collection, preservatives such as formaldehyde are usually added to the sample to ensure that the analytes are not adversely affected by microbial activities. Typically, aqueous samples are filtered or centrifuged to remove suspended particles that may interfere with the extraction procedure. Glass fiber filter paper with pore sizes between 0.22 and 1.20 μ m are commonly used for filtration in estrogen method development. Collected samples are typically refrigerated at 4 °C in amber bottles until analysis. For accurate results,

extraction is generally carried out within 72 hours after collection.^{14,17}

Sample Extraction: Pre-concentration of a large sample volume into a smaller volume is essential to reach demanded detection limits. Initial sample volumes ranging from 100 mL to 2.5 L were used in previous studies.¹⁴⁻¹⁸ Classical sample pre-concentration approaches such as solvent sublation, steam distillation, and liquid-liquid extractions were commonly utilized in the past. These techniques have been replaced by more efficient and versatile solid phase extraction (SPE) techniques.^{14-18, 23-36} Other approaches such as solid phase micro extraction (SPME)³⁷ and ultrasonic extraction³⁸⁻³⁹ are also used in environmental estrogen analysis to a lesser extent. These approaches use much less organic solvent than the mentioned classical methods.

Solid Phase Extraction (SPE): SPE operates either on disks or, more commonly, on disposable cartridges. Even though disks reduce sample clogging and have a larger surface area for sample contact compared to cartridges, they require larger volumes of solvents for analyte elution which increases the overall method duration whilst the eluted sample is blown down.¹⁴ Reversed phase SPE cartridges such as octadecyl bonded silica (C-18),²³⁻²⁷ graphitized carbon black (GCB) cartridges such as carbograph cartridges,³⁶ and polymeric cartridges such as strataX⁴⁰ and most commonly, hydrophilic lipophilic balance (HLB)²⁸⁻³⁵ are used in estrogen extractions.

A solid phase extraction procedure consists of five steps, (1) wetting the sorbent (open bonded packing material via solvation), (2) conditioning of the sorbent (treating it with a solvent that is as "sample-like" as possible), (3) loading the sample, (4) rinsing or washing the sorbent to elute extraneous material (using a solvent in which the target analyte is insoluble), and (5) elution of the analyte of interest (using an elution solvent in which the target analyte is highly soluble).⁴¹⁻

⁴³ For each step, appropriate solvent, volumes, and flow rates should be optimized for best results. For HLB extractions, methanol (MeOH), acetone, and acetonitrile (ACN) are commonly used as eluents.²⁸⁻³⁵

SPE extract is subsequently blown to a further reduced volume using a gentle nitrogen stream. It is obvious that when a large volume of environmental water sample is concentrated to a very small volume, a considerable amount of humic substances and other unwanted matrix components are also concentrated into the extract. Therefore, methods with more sample volumes often result in higher matrix interferences.⁴⁴

Sample Clean-up: Extracts from heavily contaminated water samples often require additional sample clean-up step(s) to reduce co-extractives in the

extract. A typical cleanup method requires an additional SPE step using polar cartridges such as silica,⁴⁵⁻⁴⁷ NH₂ (silica based basic bonded phase),^{29,46,48-49} or florisil (activated magnesium silicate bonded phase) to eliminate the remaining matrix interferences. C-18 SPE has also been used in further clean-up steps.⁴⁸ Sample clean-up steps help to obtain reduced matrix interferences and high signal to noise ratios (SNR).¹⁵

Methods for residue estrogen analysis in water samples without further clean-up steps are rare in literature. For example, Kuch *et al.* (2001) reported an analysis of estrogens in surface water, drinking water and wastewater by GC-ECD and GC-MS with no further clean-up steps. In the GC-ECD study, they observed significant unidentified peaks that interfered with analyte signals.⁵⁰

Derivatization: Derivatization of the analyte prior to chromatographic analysis can benefit a determination in multiple ways. In general, sample derivatizations are carried out mainly, (1) to improve the detectability of analytes, (2) to prevent analyte decomposition during chromatographic analysis, (3) to improve the chromatographic behavior of target compounds, (4) to improve the resolution of a chromatogram, (5) to establish the analyte identity, (6) to improve selectivity of analytes in a complex matrix, (7) to achieve better chemical and physical properties of analytes for chromatography (volatility, solubility, mass), and (8) as an additional clean-up step. Selection of the derivatization for a method is very important as every derivatization consists of several manipulations, which can be potential error sources. Blank derivatizations should be carried out to ensure the accuracy of the results and ultra-pure chemicals should be used to avoid contamination.⁵¹

Gas chromatographic (GC) determinations of thermolabile, polar, and low volatile compounds such as estrogens require a derivatization step to avoid thermal decomposition, improve the chromatographic separation and the sensitivity of analysis. Liquid chromatographic (LC) derivatizations of estrogens are mainly carried out to enhance detector sensitivity (Ex: attachment of a chromophore to improve UV absorbance in HPLC-UV, attachment of a fluorophore to enhance fluorescence intensity in HPLC-fluorescence).^{14-18,51}

Instrumental Analysis

Chromatography Hyphenated with Mass Spectrometry: Among the different chromatographic techniques used for the analysis of estrogens in environmental water samples, the most widespread are chromatography hyphenated mass spectrometric

techniques such as GC/MS, GC/MS/MS, LC/MS and LC/MS/MS. Chromatography separates chemical components in a mixture based on their volatility (in GC) or affinities for the stationary phase and mobile phase (in LC). Conventional chromatographic detectors (UV, fluorescence, etc. in LC and TCD, FID, NPD, etc. in GC) primarily qualify substances based on retention time and quantitate substances based on peak intensity and peak area. In contrast, MS detectors offer a highly sensitive detection based on their mass-to-charge ratios (m/z) and measure the intensity of each ion.

Scan, SIM, and MRM Modes of Mass Spectrometry: MS is extremely helpful for qualitative analysis as it can indicate peak areas of ions that have a certain mass. Yet, this only applies when measuring a single component at a given retention time as MS m/z data become useless if multiple components are simultaneously eluted. In contrast, Selected Ion Monitoring (SIM) provides excellent results in quantitative analysis when the m/z values of target analytes are known.

SIM detects and plots only the selected masses of target compounds. In SIM the MS is set to scan over only a very small mass range around the anticipated m/z values of the analytes, typically one mass unit. Narrower mass ranges provide more specific SIM assays. This enables to separately quantify compounds even if they have the same retention times. In other words GC or LC-SIM-MS methods provide a two dimensional separation based on the retention times and the m/z values of target compounds in a complex mixture. SIM mode MS dramatically increases the signal-to-noise ratios (SNR) of desired peaks compared to full scan MS resulting in low detection limits. Also SIM methods provide more selectivity to the analysis as only chosen m/z values are scanned. This results in reduced matrix interferences in the chromatogram.

The introduction of tandem mass spectrometry hyphenated with chromatography (LC or GC/MS/MS) has substantially improved the detection limits and enhanced analyte identification. Multiple reaction monitoring (MRM) methods used in tandem mass spectrometric (MS/MS) techniques provide further improved selectivities as two desired ions are monitored for a given compound. In MRM technique, the parent mass of the compound is specified for MS/MS fragmentation by the first MS and then a selected fragment ion is monitored by the second MS.⁵²

LC/MS and LC/MS/MS Methods in Residue Estrogen Analysis: Octadecyl silica stationary phases are generally used in LC separations of estrogens. Water/ACN mixtures with gradient elution from 20 to

70% ACN have commonly been used as mobile phases. Electrospray ionization (ESI) is the most extensively used LC/MS interface. Electrospray ionization has been utilized in both negative [ESI (-)]^{30, 34-35, 45, 53} and positive [ESI (+)]^{28, 47} modes. However, atmospheric-pressure chemical ionization (APCI) is also used to a lesser extent.^{31, 54} Most common mass analyzers for estrogen analysis are quadrupole mass analyzers. The use of triple quadrupole (QQQ) mass spectrometers in residue estrogen analysis has considerably enhanced the selectivity and sensitivity of the determination, resulting in improved detection limits than those achieved by use of single quadrupole LC/MS.^{15, 17-19, 32, 37}

GC/MS and GC/MS/MS Methods in Residue Estrogen Analysis: A variety of capillary columns have been utilized for GC separations of estrogens in environmental samples. Splitless mode 1 to 4 μ L sample injections were extensively used in analyses. In most studies, helium has been used as the carrier gas, with temperature programs ranging from approximately 45 to 300 °C. Electron impact (EI) ionization is most commonly used in GC/MS methods.^{29, 33, 49, 55} Quadrupole mass analyzers were frequently used in conventional MS, while triple ion trap MS is widely used in tandem mass spectrometry.^{33, 49, 55}

A flow diagram of a typical analytical approach to determine residue estrogens in environmental aqueous samples is shown in Figure 1.

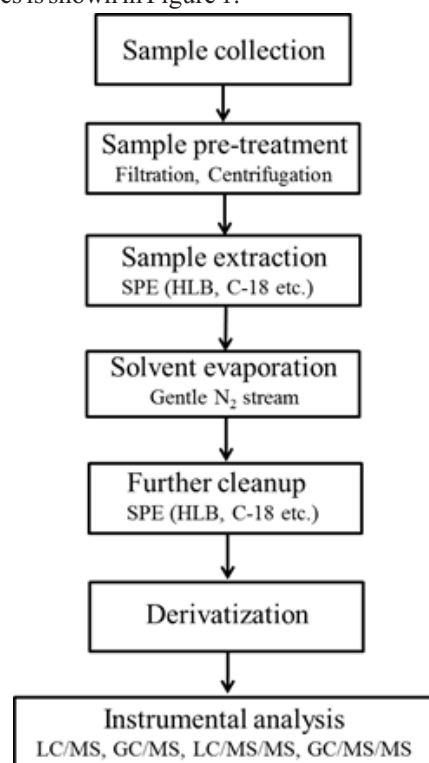


Figure 1: A flow diagram of a typical analytical approach to determine residue estrogens in

environmental aqueous samples.

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## Ion Mobility Spectrometry: An Economical Analytical Technique

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An ion mobility spectrometer is a simple device that can be constructed with a few electrodes, ceramic rings and resistors, yet it has become the method of choice for many trace organic analyses. Ion mobility spectrometry (IMS) is often used in military and security applications for detection of narcotics, chemical warfare agents, toxic industrial chemicals and explosives at the parts per billion levels. During the past two decades, IMS has been extensively developed as an in-expensive, fast and reliable separation and detection technique; coupled with multidimensional modes such as mass spectrometry, liquid chromatography and gas chromatography, it can provide analytical advantage to these methods not possible with stand-alone instruments.

The IMS was developed on the basis of fundamental experiments of gas phase ionization and ion mobility behavior conducted by Rutherford, Thomson, Tyndall and Townsend. These early IMS experiments were conducted under low pressure. Cohen et.al. demonstrated that IMS could be accomplished under atmospheric conditions. The ambient pressure ion mobility spectrometry was first known as 'plasma chromatography' and was used as a detection and peak characterization method coupled to

gas or liquid chromatography. Discoveries of ion molecular reactions in ion mobility have expanded the capabilities of IMS lately. Non-radioactive ionization sources (eg. corona ionization and photo ionization) successfully employed in IMS which became useful in constructing some portable IMS devices. Hand held ion mobility spectrometers have started to develop in late 1980s which were first used in military zones for monitoring chemical weapons. Several IMS devices, capable of monitoring single or limited number of analytes was then developed for airport check points and air pollutant monitoring. Some portable IMS were also constructed by coupling with gas chromatography which acted as a pre-separator when handling complex samples.

### 1.1 Theory

IMS separates and identifies analyte ions based on their mobilities while they are traveling under the influence of a constant electric field in a drift tube with a counter-current buffer gas flow. Mobility separation in IMS depends on the collisional cross sectional area between analyte ions and the drift gas molecules. Mobility of an ion drifting through a constant electric field is defined as

$$K = \frac{v}{E} \quad (1)$$

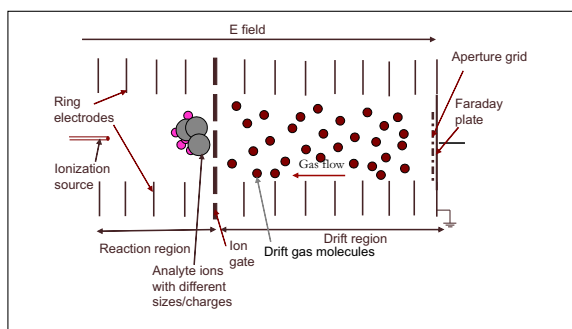
where,  $v$  is the velocity of ion,  $E$  is the applied electric field and  $K$  is the mobility constant of the ion. Mobility can be correlated to the system parameters as,

$$K = \frac{L^2}{Vt_d} \quad (2)$$

where  $t_d$  is the time the ion takes to travel through the drift region,  $V$  is the applied voltage at the beginning of drift region and  $L$  is the length of the drift cell.

## 1.2 Instrumentation

A basic ion mobility drift tube is constructed by alternatively stacked ring electrodes and ceramic washers. Figure 1 illustrates a schematic diagram of a cross section of typical stand-alone ion mobility spectrometer. Voltage is applied to the first electrode of the IMS tube. All the electrodes are electrically connected to each other with a resistor series so that a linear voltage gradient is maintained along the IMS tube. Counter flow of a drift gas (eg.  $N_2$ , Air, or  $CO_2$ ) is maintained in order to clean the drift tube from the neutral sample and contaminating molecules. Ions are introduced to the tube from an ionization source in the ionization region. The ion gate, which is located after the ionization region, is pulsed open for a short time interval (eg. 50-200  $\mu s$ ) by a gate driver in order to initiate the ion mobility spectrum. Short pulses of ions are then transferred to the drift region along the voltage gradient. Mobility separation occurs while the ion pulse drifts through the drift region, separating the ions according to their size and charge.



**Figure 1:** A cross section of a typical stand-alone ion mobility spectrometer

At the end of the drift region ions are collected by a Faraday plate in standalone IMS. Instead of having a Faraday plate, IMS tube also can be coupled to a mass spectrometer, which known as ion mobility-mass spectrometry (IMMS) in order to obtain the mass information of the detected ions. IMS typically operates at ambient pressure and elevated temperature of 150-200 °C.

### 1.2.1 Ionization source

Ionization is an essential process in IMS in order to produce gaseous ions from the neutral sample analytes so that the resulting ions can be focused under an electric field for ion mobility separation in the drift region. The primary ions produced in the ionization process are known as reactant ions in IMS. Most commonly used ionization methods in IMS are electrospray ionization,  $^{63}Ni$  ionization and corona ionization.

Electrospray ionization is a non-radioactive ionization method. Voltage is applied to the liquid sample which delivered to the ionization region of the IMS through a capillary tube. A target with opposite voltage is positioned in front of the capillary tube exit where the sample with the voltage enters to the atmosphere as charged droplets. While the charged droplets flying towards the target the solvent droplets convert to naked ions. According to the solvent evaporation modal the solvent that surrounding the ions get evaporated gradually until the droplet increases its Coulombic interactions and finally undergoes a fission process to produce single or multiple charged ions. In ion evaporation modal this explains that the ions get ejected from the surface of the solvent droplets and ultimately produce tiny droplets of charged species. In  $^{63}Ni$  ionization beta radiation is produced by  $^{63}Ni$  foil (1cm× 3cm) that can undergo series of atmospheric reactions to produce the reactant ions. Corona ionization is produced by applying voltage to the sharp needle tip, which can ionize the neutral atmospheric gases, such as  $O_2$ ,  $CO_2$ , and  $NO_2$  to create the reactant ions. Commonly found reactant ions are  $H^+(H_2O)_n$  in positive mode and  $O_2^-(H_2O)_n$  in negative mode.

### 1.2.2 Gating

The ion gate can be periodically opened and closed allowing short pulses of ions introduced to the drift region. Efficient gating is important in order to reach the maximum sensitivity and sharp peaks. Two commonly used ion gates in IMS are the Bradbury Neilsen gate and the Tyndall gate. Both gates have two sets of shutter grids. In Bradbury-Neilsen gate the two sets of grids are arranged coplaner while in Tyndal gate the two sets of grids are in different planes, parallel to each other in few millimeter distance. When a pulse width is applied, the gate opens to let the ions drift for short period and then a gate potential changes to create an E field in the opposite direction and stops the ion swarm from entering the drift region during the remaining run time.

### 1.2.3 Ion detection

For stand-alone IMS instruments, a Faraday plate is normally used to detect the ions as ion current passing through the drift region of the spectrometer. As an ion swarm drifts through the tube, a charge can be induced on the Faraday plate. This is called mirror current, which leads to reduced resolving power by superficially broadening width of the ion swarm. This problem can be resolved with an aperture grid located before the Faraday plate to electrostatically shield the Faraday plate from the charge of the approaching ion swarm.

### 1.3 Miniaturized Ion Mobility Spectrometers

In order to minimize cost, power, and weight for analytical instruments, size reduction of instrumentation for on-site detection of harmful chemicals is desired. Unlike mass spectrometer, ion mobility spectrometer works at atmospheric pressure and therefore no vacuum system is required permitting IMS operating as a portable device. Several compact IMS devices with 1-5 cm long drift tubes have been constructed and evaluated. These include resistive coated ceramic tubes, integrated stack electrodes, and ceramic washers having improved electric field uniformity. In addition mini scale glass fabricated devices and ion focusing aspiration systems have been developed. Today, many commercially available ion mobility spectrometers used in environmental, medical, and military applications have been reduced in size such that they can be used as portable devices.

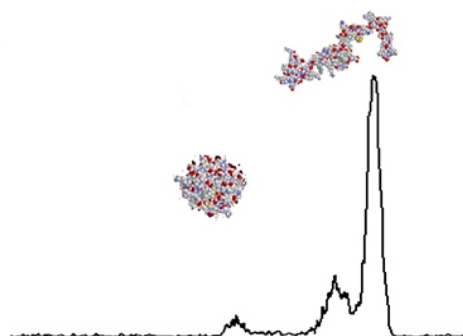
### 1.4 Applications of Ion Mobility Spectrometry

Law enforcement agencies use ion mobility spectrometers with the response time fast as few milliseconds to detect trace explosives in security checkpoints and airports and war zones. Part per billion concentration sensitivity has been achieved detecting trace explosives such as trinitrotoluene (TNT) and cyclo-1,3,5-trimethylene-2,4,6-trinitramine (RDX).

IMS has been also popular as a sensor for toxic industrial chemicals in nanoscale levels. In addition it has been useful in detecting pesticides and herbicides in fruit surfaces, with minimum sample preparation steps.

IMS have been applied to characterization of food products and process control such as beer fermentation and wine production. In addition, IMS has been capable of use as technical tool in food quality control. ng/L detection limits were helpful in obtaining extremely sensitive odor limits. For example, control and identification of mold growth in dairy products. Furthermore IMS has been also used for quality control of packaging materials.

IMS have been useful in forensic applications. It is capable of detecting illicit drugs, trace elements of chemical warfare agents, explosives related in forensic studies. IMS has also been utilized detecting illicit drugs in hair and sweat samples.



**Figure 2:** Example mobility separation for the  $[M+8H]^{8+}$  ion of cytochrome *c*. As shown here, ion conformations that are more elongated have lower mobilities and longer drift times than that of the shorter ion conformation. <http://www.indiana.edu/~clemmer/Research/Intro.php>

One important advantage of IMS is when coupled to mass spectrometry isobaric ions, which are ions with same mass can be differentiated due to their different mobility values. This is essentially important in analyzing complex biological samples in metabolomics. Figure 2 illustrates an example of an ion mobility spectrometric data obtained for two different conformations of cytochrome *c* separated based on their shape. Ion mobility coupled to mass spectrometers (IMMS) have been successfully used for peptide sequencing, protein identification, analysis of carbohydrates and inorganic compounds. Furthermore IMS data have been utilized to identify various petroleum products from crude oil samples. Since IMS has the ability to identify structural isomers it can provide some extra information than the high resolution mass spectrometer.

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Honorary Rector of College of Chemical Sciences

Professor Samitha P Deraniyagala, Senior Professor in Chemistry at the Department of Chemistry, University of Sri Jayewardenepura was appointed as the second Honorary Rector of the College of Chemical Sciences by the Council of the Institute on the recommendation of the Academic Board with effect from 6th March 2015. Professor Deraniyagala has served as the Faculty Head of the College in 2008 and the President of the Institute from 2009 for two consecutive years.

Benevolent Fund Benefits for Members

i. **Long life benefits:**

Amount provided will be as follows:

a. Over 70 yrs : Rs. 12,000

b. Over 75 yrs : Rs. 18,000

c. Over 80 yrs : Rs. 25,000.

ii. **Critical illness benefits:** up to Rs. 60,000

iii. **International travel for conferences** (with presentation of a paper):

a. Passive members : Rs. 30,000 (international travel only)

b. Active members : Rs. 60,000 (international travel and/or accommodation).

Any member who has paid membership fees for life (after 3 years of such payment) is entitled for these benefits.

All members are advised to pay the membership fee for life and become beneficiaries.

Eleventh Convocation of the College of Chemical Sciences

Convocation Address

Information Knowledge and Wisdom

Professor Savithri Goonasekara

Former Senior Professor of Law, The Open University of Sri Lanka,

Former Vice Chancellor, University of Colombo.

As an academic and a member of the legal profession who is not a chemist, I feel privileged to have been invited by the Academic Board of the Institute of Chemistry of Ceylon to be the Chief Guest at the 11th Convocation of the College, held today. Professor Fernando told me many years ago, when we were foundation professors of our respective fields, Chemistry and Law in the Open University of Sri Lanka, that Chemistry was the "central science," in the field of Science. I could at that time, claim recognition for my own personal view that law was also a "central science" because, I was a Professor in the Faculty of Humanities and Social Sciences of that University. Today however there is a debate whether law can be considered a social science. Besides, there are people who think that the rule of law is a Western conspiracy against our lovely land, and the law is an ass. My credentials as a former Vice Chancellor, do not give me any sense that I am a deserving Chief Guest, since the office has been so denigrated that I would prefer to have been like your distinguished Rector, in an eminent group of Senior Professors who never held that post.

I thank the Rector and the Academic Board of the Institute of Chemistry for honouring me, and inviting me to participate and address you at this Convocation. I congratulate the Staff and students of the College on the successful completion of their study programme. We live in an era where information technology and new scientific developments are propagating the public message that education is simply a path to certification and qualification and acquisition of technical skills. Capacity for creative thought and ideas are perceived as "soft skills." Sri Lanka has also experienced many years of tensions within Sri Lanka's higher education institutions, disrupting academic programmes. We should all perhaps remind ourselves on an occasion like a Convocation and an awards ceremony, that learning involves more than information gathering. It must create a relationship of trust and confidence between student and teacher, in expanding the horizons of knowledge. A certificate and diploma is not just a qualification but a commitment to use the knowledge acquired through that important staff student relationship, and meet high standards of professional excellence, and citizen responsibility in a nation.

The tradition of learning goes back to antiquity and

the ancients, especially in Asia. This is because it has been recognized throughout human history, that learning knowledge and wisdom that can impact to improve the lives of people, are a rich national resource and all interconnected. This is why education is valued, and different cultures recognize that "in learning lies knowledge and in knowledge lies wisdom." I would like therefore to reflect on some of these concepts and their relevance to the contemporary education environment in Sri Lanka today.

Information Technology has revolutionized learning and the acquisition of knowledge. However the challenge in Sri Lanka today is to ensure that these path-breaking and creative developments do not contribute to an erosion of respect and regard for past experience. The traditions of learning of the past, tell us, that accumulating information, whether by memorizing or by Google searches and access to the internet, cannot help to impart knowledge or wisdom. There must also be an opportunity for thought and reflection, discussion and debate with teachers and scholars. Experience also tells us that study, research, and scientific knowledge, if they are to be used for human wellbeing, must be connected to some ethical standards and values on how we use knowledge and learning with a sense of social responsibility. When we look back on Sri Lanka's own experience in higher education, we see that in recent decades we have expanded opportunities to acquire learning, but often forgotten in the process the need to focus on the wider goals of education in imparting knowledge and wisdom.

In that context, this College of Chemical Sciences, of which you have been privileged to be students and Staff, has been founded on principles that suggest that this institution has succeeded in making this important linkage. The CCS Newsletter, Vol 13 No 5 shared with me gave a cameo insight into the broad range of your activities and programmes. It shows that the discipline of chemistry is not viewed narrowly, but linked to general issues of public concern, like environment -a topic that requires interdisciplinary responses. Most importantly the first page highlights a normative and ethical value, when it declares that "education is not just filling a basket but lighting a fire." What better way to encourage all of you, the new graduates and

diplomates, to go forth from the College into the outside world, with a passion and commitment to use the professional skills and knowledge you have acquired, in service to your profession, your community and your country.

The College of Chemical Sciences has perhaps been more fortunate than some others in this country in being anchored in a professional institution with a long and distinguished history. The Institute of Chemistry is perhaps one of the few Sri Lankan Institutions that retains in its name, a reference to our colonial identity, as "Ceylon." The Institute in itself is a successor to the Chemical Society of Ceylon founded in 1941. The road on which the College is located, is called Professor M U Sultan Bawa Mawatha - honouring a distinguished Sri Lankan chemistry professional and academic of the past. The College is therefore a new institution linked to a distinguished past. It has also pioneered the concept of providing education opportunities outside the monopoly of a State system - representing an important and creative experience in public and private sector partnership in education. I hope that as new graduates and diplomates, you will not forget the importance of that legacy, and you will be the kind of professional who will carry forward the best professional traditions of the Institute of Chemistry.

A Convocation is also a time to reflect on the institution builders who have created the education environment for your studies. When I walk down memory lane, I can recall the passion and commitment with which Professor J N O Fernando, your Rector, first mooted his idea of providing a parallel path of entry to your profession through a School of Chemistry located in the Institute. I am personally aware how difficult it was at the time to gain recognition for the novel idea of Non-State and private institutions becoming centres of excellence in higher education. Professor Fernando dreamt a dream, and gave leadership within the Institute, to realize a personal dream that was also located in an ideal of service to his profession and his country. Many of us dream dreams, but do not have the dynamism and tenacity to push the barriers and expand frontiers to achieve our dreams. The pioneering achievement of this College in creating a new path in professional education in over four decades must be a source of pride to each one of you, and also guide you in your own professional endeavours and careers.

One of the challenges that will face you, as you move into the world of work and professional engagement, will be to combat the growing "de-professionalisation" that has been enthroned in this country in the name of development. The pressure for quick and swift solutions, once again linked to the barrage of swift solutions provided through information

technology and access to the internet, suggests that non-professionals can do the job of professionals as cleverly and excellently. In the last decade in particular, the myth that professionalism is old fashioned and antiquated has had a profound impact on the perception among policy makers that they really do not need professionals. This in turn has created a context and environment where politicians in the driving seat use the imperatives of politics in making key development decision that call for professional advice. I hope that changes in that view of national development will be initiated in the now famous 100 days plan, and continue in the years to come. You as new graduates and diplomates will hopefully then not encounter the barriers and constraints that others faced in the recent past.

However it is also important for you to understand the personal responsibility of professionals to resist erosion of professionalism in our country. Private institutions must conform to State policies that are conducive to national development, but also engage actively in responding to and criticizing negative trends in the economic political and social arenas that ultimately do impact on one's capacity to perform, and do one's job. Sometimes passivity and being uninvolved creates acceptance for negative changes that can destroy both a profession and a nation.

It is in that context that I would like to suggest that core values and principles on the rule of law are key to professional development and the practice of a profession, and the very functioning of institutions. When breaking the law and dismissing the concept of legal regulation as a barrier to progress is accepted, the public interest is ignored, and every one of us has to accept that arbitrary and unreasonable decisions which impact on our lives can be made by anyone exercising power and authority. We lawyers say that there is a science of legal norms and standards developed through centuries of experience in controlling abuse of power. "The rule of law" is therefore not a Western imported colonial concept, but a set of standards developed through human experience on containing abuse of power. Some of that experience came in our country from our colonial past, and some from our own historical experiences. Many of those values and standards have been incorporated in the Chapter on Fundamental Rights in the Constitution of our country, as well as in the first independence Constitution of India, and other South Asian countries. So we as citizens and professionals from disciplines other than law cannot afford to dismiss ideas on the rule of law as either alien to our culture, or unscientific and old fashioned values that need to be rejected as we pursue an aggressive path to social and economic progress for

our people. A chemistry teacher and a student focus on the laboratory as the source of learning, knowledge, and wisdom. Law teachers and students will argue that the library in our laboratory, and that though we do not conduct experiments in our laboratory, we can access the wealth of human experiences that throws light on the past, and also creates new awareness of how to respond to future challenges in our social political and economic environment.

I am not sure whether the academic programmes of this College have any components that deal with the nature of your professional responsibilities in the context of law and regulation. I see this as a real need in an environment where we have created myths about the need for less laws or no laws to encourage national progress. The barrage of recent information in the media on corruption and illegal use of power remind us that the public interest has suffered from both lack of a law regulating the citizen's right to information, and even the self censorship of professionals and the media in the face of corruption and abuse of power. Perhaps

courses on a citizen's responsibilities under the law and the Constitution, and practical insights about how the absence of law encourages abuse of power and impacts on our lives, should be perceived as important to becoming responsible citizens and good professionals. You are all graduating today with a qualification that brings with it new hopes and aspirations and opportunities. You will also perhaps qualify to be members of a professional institute that has had a long and distinguished history. You are also graduating at a time when important changes are taking place in the political economic and social fabric of our nation. I call upon you to use your knowledge with wisdom, and insight, with passion and commitment to use your knowledge and contribute to the well being of our society. It is important to remember that you have role models in the founders of this Institution who dreamt a dream of creating a centre of excellence in Higher Education for Chemistry professionals, and gave dedicated service to create this Institution for you.

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*(Report of the Honorary Rector presented at the Eleventh Convocation held at Eagles Lakeside Banquet & Convention Centre on 19.02.2015)*

**A Fantastic, Unique, Historical, Unbelievable and Proud achievement:  
CCS produces 1075 Graduate Chemists and 1025 Chemistry Technicians  
through a high quality professional programme at the lowest possible cost  
with no delays.**

Late Professor J N Oleap Fernando, *C.Chem, C.Sc.*

*Former Honorary Rector and Honorary Professor, College of Chemical Sciences, Institute of Chemistry Ceylon.*

We celebrated the completion of 40 years of professional chemical education, the Ruby Anniversary of our Technician Diploma in Technology in Chemistry Programme, with the holding of a very successful International Conference on Chemical Education on 3<sup>rd</sup> and 4<sup>th</sup> April 2014.

Having being involved with the **Diploma in Laboratory Technology in Chemistry (DLTC) Programme** from its inception in 1973, it gives me utmost pleasure to report to the eleventh convocation that the 40<sup>th</sup> DLTC batch who will formally receive their diplomas today comprise the highest number ever of **80 diplomates** pushing up the total production to a magnificent **1025**. May I once again remember with gratitude our forefathers and in particular Dr. Sente Shanmuganathan (then President, IChemC and founder coordinator) for having had the foresight, forethought and altruistic sense that enabled a serious lacuna at the middle level in Chemistry to be thus filled. We have just enrolled the 42<sup>nd</sup> batch of **115** students and this two year programme continues as the only such

programme producing technicians in any of the basic science disciplines in Sri Lanka. May I also mention that the Council of the Institute has decided to confer on Dr. Sente Shanmuganathan an Honorary Fellowship of the Institute at the Annual Sessions in June.

**The Graduateship Programme in Chemistry** (that was commenced in 1979) has also proceeded with much vigour and expectation and we have just registered the 37<sup>th</sup> batch of **206** students for this four year programme. It gives me supreme pleasure and joy to also report that no less than **106** Graduate Chemists are passing out today as the 32<sup>nd</sup> batch and thereby concurrently increasing the overall total production of Graduate Chemists by the Institute of Chemistry Ceylon to a gigantic total of **1075**. We are thus now producing **50%** of the Graduate Chemists produced in Sri Lanka equalling the total production of Special Degree Chemists by the 6 other Sri Lankan Universities offering a similar programme.

Our professional body of Chemists in Sri Lanka

can be justly proud of this Fantastic, Unique, Historical and Unbelievable achievement of producing a total of **1075** Graduate Chemists and **1025** Chemistry Technicians and thus meet the needs, demands and aspirations of a very large number of Sri Lankan students, many of whom would otherwise have had no such opportunity. We have thus satisfied the late developers, the late realisers, adults, matured students, middle level employed persons and in more recent years plenty of school leavers to make use of our endeavours in human resource development in the area of Chemical Sciences.

Our programmes are unique in many ways and if I may enumerate some of them:-

- i) We are probably the **only** professional body in the world to provide such a formal tertiary level educational programme in Chemistry.
- ii) Our Graduateship Programme is the only such programme in Sri Lanka to receive international accreditation (by the Royal Society of Chemistry in the UK)
- iii) Our programmes are guaranteed to be completed within the advertised periods of study.
- iv) We are able to make use of the best available resource persons from amongst the numerous universities, research and service institutions, private sector etc and thereby provide our students with a unique & wonderful opportunity.
- v) In terms of cost, the total cost for a student entering the programme today is of the order of 4.5 lakhs (for the entire 4 years Graduateship Programme) and rupees 1.25 lakhs for the entire 2 year DLTC programme. These are undoubtedly the lowest of the costs that would be incurred to follow a similar programme in any part of the world.

The 1075 Graduate Chemists produced so far constitute over two thirds of the membership of the Institute of Chemistry Ceylon. Our alumni are life members of the Institute and are thereby not only joint owners / stake holders of whatever the Institute possesses but are also direct beneficiaries of the **Institute's Benevolent Fund** and the more recently established **Graduate Chemists Welfare Fund**. No similar benefit is available to alumni of our Universities/higher education systems.

From the national point of view, we have thus provided the services of 2100 Graduate Chemists and Chemistry Technicians at no cost whatsoever to the national exchequer. Taking into account a conservative estimate of what the national budget spends on the free education of a typical tertiary level science student for higher education, our notional contribution to this magnificent human resource development endeavour

is well over Rs. 3 billion rupees (USD 225,000) over the past four decades. This is indeed no small contribution to the nation from a professional body such as ours.

#### Adamantane House

We are thankful to the UDA for the provision of a 25 perch block of land to put up our own Institute building (Adamantane House) in 2005. With the extension put up last year, we have virtually doubled our space availability to about 20,000 square feet with the consequence that we now have an Auditorium, 5 other lecture halls, 2 undergraduate laboratories, a research laboratory, Instrument room, an expanded library, a new Board Room equipped with all facilities, many staff rooms, a pent house on the rooftop and of course, plenty of toilets. **We probably are the only institution that has put up a building on UDA leased land which has not leased out a single square foot of built up space in order to obtain an income.** We have made use of all the possible space for the benefit of our students and members. I therefore have once again to express my great regret that all our efforts to obtain an additional block of land nearby has not yet borne fruit although the required preliminary advance for an **allocated block** nearby was paid to the UDA seven years ago. It is indeed a sad state of affairs when a body such as ours who are ever ready to use such space to further human resource development without ever obtaining an income from it like others has been unfairly, unethically deprived of such an opportunity. We continue to rent out square feet of space from the College of Surgeons in order to have more room for staff and student Common Room.

#### Academic Staff

Professors M D P de Costa and Srianthie Deraniyagala are currently spending their sabbatical leave while serving as Senior Professors. Professor Costa is also functioning as Dean and providing much needed support to the College. Professor Deraniyagala delivered the (seventh) Inaugural Professorial Oration on the 5th of February. With the recent appointment of another of our own Graduate Chemists, Dr. Ranmal Gunathilake, as a full time academic, the total number of full time academic staff increased to 13 and this includes 6 of our own Graduate Chemists. We also have five other part time academic staff – Professor Priyani Paranagama, Professor K A S Pathiratne, Dr. Vinitha Thadhani, Graduate Chemist Dr. Dinara S Gunasekera & Dr. Viraj Jayawardena – giving an overall total of 18 academic staff members which is the highest on record. With the additional assistance of 33 (full time and part time) Teaching Assistants and a large number of

visiting lecturers our College has been able to enable our two educational programmes to proceed with greater speed, quality and efficiency.

#### **Duplication of both programmes on week-days**

For example, we are now offering Level 2 of the Graduateship Programme also on three week days with the result that the entire DLTC Programme and the first 2 years of the Graduateship Programme are now duplicated on week days, in addition to the customary week end programme. The (compulsory to sit) advanced courses at Level 3 are now repeated every year while more optional courses are being offered at Levels 3/4 on a once in a two year basis.

#### **Stand-alone Certificate Course in Food Chemistry and Technology**

This three credit Level 3 / 4 course was offered to anyone interested during the course of the past academic year and we are glad to report that 6 students successfully completed this course. We intend to offer more of such stand alone certificate courses (such as Separation Sciences) provided there are adequate numbers interested.

#### **Non-Academic Staff**

The non-Academic staff members have also increased in order to cope up with the work at hand. Together with the recent appointment of Additional Registrar, Senior Assistant Registrar, Deputy Librarian, Internal Auditor and two Assistant Librarians the total non-academic staff number has increased to 22.

The total number of regular staff (academic and non-academic) working at Adamantane House has thereby increased to over 70.

#### **Research & Instrumentation**

One of the principal deficiencies of our academic programme until we moved to our own premises – Adamantane House – in 2005 was the inability to conduct any type of research work. From 2006, we have gradually increased and enhanced research activities in the College and an increasingly large number of students are pursuing undergraduate research projects at Levels 3 / 4. Those who are unable to do so are now required to offer at least a literature survey course or be involved in a seminar presentation.

We have also increased the number of college funded Research Assistantships to 5 in order to enable peer reviewed research projects formulated by our internal staff to be conducted in a more positive manner leading to postgraduate degrees.

Coupled to these research projects and otherwise,

we are gradually increasing the number of sophisticated instruments available for post graduate as well as undergraduate research/ class work. The availability of a dedicated research laboratory and an Instrument Room has greatly assisted this process.

#### **Co-Curricular Activities**

Debating continues to be an important co-curricular activity both on an inter-level basis as well as on an Inter University basis.

The following co-curricular lectures were held for the benefit of our students.

- (1) Professor Atta Ur Rahman (from Pakistan) on 2<sup>nd</sup> April 2014 on “The Wonderful World of Chemistry”
- (2) Dr. Paul D Lickiss (from UK), Graduateship External Examiner on 5<sup>th</sup> April 2014 on “Adventures in Silicon Chemistry”
- (3) Dr. Ananda Seneviratne (from USA) on 20<sup>th</sup> December 2014 on “Analytical and Formulation Development of a Novel Class of Anti- cancer Therapeutics, Antibody Drug Conjugates (ADC)-Targeted Missile”
- (4) Professor Eugene de Silva (from USA) on 20<sup>th</sup> December 2014 on “Chemistry and You- A marriage Made in Heaven?”

#### **Extra-Curricular Activities**

The College has continued to encourage the Student Association and the students to conduct and participate in co-curricular and successfully extra-curricular activities in order to enhance their soft skills including leadership, sporting, communication, social and organizational skills as well as display their hidden talents in a formal manner. These activities included AURA-2015 (talent show), religious activities, social action programmes, Rotract Club, Cricket, Rugger, Badminton, Basketball, Martial Arts (Karate) etc. The College annually provides a substantial amount of funds towards these activities.

#### **Corporate Social Responsibility**

Following with our assistance to the SLAAS in the previous year, we have this year offered funds to the National Academy of Science of Sri Lanka to enable it to produce a better and more colourful newsletter.

We have also decided to utilize some of the funds left over from the Ruby Anniversary Celebration last year towards enabling Chemical Societies of some of our Universities to launch a mutually acceptable project/programme in the Chemical Sciences.

#### **Training Seminars/Workshops**

The follows training seminars/workshops were

organized by the College during the past year.

- (1) Seminar on “Indigenous Medicine: Role of Chemists and Recent Advances” on 21<sup>st</sup> May 2014
- (2) Seminar on “Sustainable Utilization of Sri Lanka Bio Diversity: The Role of Chemist” on 21<sup>st</sup> June 2014
- (3) Seminar/Workshop on “Teaching, Learning and Assessments” on 16<sup>th</sup> December 2014

### Monographs and CCS Publications

We have continued to publish more monographs at various levels to enable students as well as the general public to become more knowledgeable and informed. The monograph published this year was on “Organosulphur compounds in Nature” (No.33) – by Professor S. Sotheeswaran

Monographs on “Atomic Absorption Spectrometry” (No.27) – by Professor K A S Pathirathne, “Natural Toxins and Foodstuffs” (No.5) – by Professor Jans and Maduka Dias de Lanerolle and “Life & Metals” (No.19) by Professor Janitha Liyanage were reprinted on earlier stocks were over.

### Acknowledgements

We wish to acknowledge with grateful thanks the receipt of an increasing number of prizes/Scholarships at various levels of the Graduateship Programme. These include

- (1) **Mr. T Kandasamy**, Hony FICChemC, Past President (presently in Canada) who has donated Rs. 120,000/= to endow TWO prizes for the Graduateship Programme named as **President 1979** award for Industrial Safety, Health and Environmental Technology and **Lakshmi Award** for Chemistry of Gem Minerals and Synthetic Gem Materials.
- (2) **Mr. S K Cyril**, for the Graduateship Prize for Petroleum and Petrochemicals.
- (3) **Dr. Senthe Shanmuganathan**, Hony FICChemC, Past President, Ichem C has donated Canadian \$ 10,000 to establish TWO scholarships for needy students to be used towards accommodation costs.

### Our Joy and Happiness

Reports received from local and global sources indicate how well our Graduate Chemists are performing in the respective spheres of work whether it be employment, post-graduate studies or career redirection.

With increasing numbers of Graduate Chemists pursuing post graduate studies, we estimate that nearly 40% of the relevant graduate chemists cohort, who had the required time to do so have obtained post graduate degrees About 100 have obtained PhD degrees from a

number of countries including Sri Lanka, UK, USA, Canada, Ireland, France, Italy, Switzerland, Norway, Germany, Singapore and Australia. Recognizing the number of graduate chemists who are presently reading for post graduate degrees worldwide, we are confident that the number who will similarly qualify will increase exponentially over the next couple of years. It gives the Institute and the College a great deal of satisfaction and pride that our tertiary level programmes which were started in such a small way with hardly any vision or such expectations could have achieved such a status.

In this connection, we note with great pride and pleasure that one of our own Graduateship alumni from the very first batch will take over the Presidency of our Institute of Chemistry Ceylon in the Council year 2015/16 with effect for 1<sup>st</sup> of July 2015. **Mr. K R Dayananda**, who will take up this role followed the Technician Programme as well as the Graduateship Programme and rose from the level of a Technician at the ITI (then CISIR) to become a Senior Research Officer. After he retired he is now functioning as a Consultant to a leading private firm. While the College recognizes this as a very significant and notable development in the history of the College, it also gives us singular joy that Mr. Dayananda will guide the Institute as its President as we keep its 75<sup>th</sup> anniversary (2016).

We look forward to more of our alumni taking up similar important roles in the activities of the Institute, CCS and the Academic Board of the College.

For the past several years we have invited some of our own past alumni (who have excelled in their respective areas of work to ceremonially inaugurate our Programmes. We also note with pleasure that from amongst our alumni, 3 Graduate Chemists have functioned or are functioning as Heads of University Chemistry Departments while one has also been a Dean of a Science Faculty. Another Graduate Chemist is currently Head/Research of the Kotalawala Defence University. None of us who were involved with our educational programmes ever expected even a few years ago that our alumni might occupy such positions of distinction and importance in our State Universities. We are confident that more of our alumni will follow suit in the years to come.

### Conclusion

We continue to go forward with confidence, enthusiasm, satisfaction and fulfillment that our alumni are doing so well that we do not need formal advertisements or paper accreditation.

I have attempted during the course of this report to show how “we have from a very small beginning been

able to convert ourselves from a very modest narrow outfit that threatened to remain as such forever into a vibrant agent to give our Institute dimension and stability”.

(I am only proudly quoting here from a recent letter recieved from our revered Past President, Dr. R O B Wijesekara)

Before I conclude, may I therefore thank everyone, academic and non-academic, who have provided us all the support, assistance and co-operate to reach our current status and position.

Thank you all for your kind presence today and for your patient listening.

## Student Corner

### Paper Chromatography

Dr. Udaya Jayasundara

Senior Lecturer, College of Chemical Sciences, Institute of Chemistry Ceylon, Rajagiriya

**Author's note:** If you read the article published in the Student Corner in last volume, you may find out I have no intention to write this article. However, due to the request I planned to write an article on Paper Chromatography.

As we have discussed in previous articles, the chromatography is primarily used to separate mixtures of substances into their corresponding components. They all have a **stationary phase** (a solid or a liquid supported on a solid) and a **mobile phase** (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Not all the components in a mixture travel at the same rate. These differential rates pave the way to separate the mixture into its components after a particular time which is called the **retention time**.

#### What is paper chromatography?

In paper chromatography, the stationary phase is a uniform absorbent paper. The mobile phase is a liquid solvent or mixture of solvents. The governing principle behind the paper chromatography is the capillary action. It is defined as the movement of liquid within the spaces of a porous material due to the forces of adhesion, cohesion, and surface tension. The liquid is able to move up the filter paper because its attraction to itself is stronger than the force of gravity. However, there are other methods which employ the solvent development with gravity.

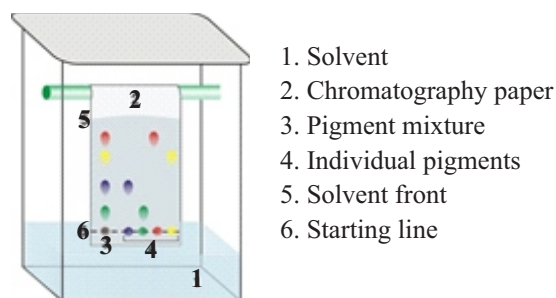
Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and the stationary phase. . Solutes dissolve into solvents that have similar properties. (Like dissolves like) This allows different solutes to be separated by different combinations of solvents.

#### How to produce a paper chromatogram?

May be this is the very first question that you would come across when you are in the organic

chemistry laboratory as this is one of the simplest experiment performed across the world. In fact, the paper chromatography is one of the first techniques you might perform in chemistry to separate out mixtures. Further, this is a simple and low cost experiment which can be completed (in most cases) within 10 to 15 minutes.

Let's think about a very simple example which can be practically performed in a laboratory. Assume a mixture with several components is spotted on the paper. The paper is suspended in a container with a suitable solvent or mixture of solvents in it. It is important that the solvent level should be below the line (see the starting line on Figure 1) with the spots on it. Sometimes the paper is just coiled into a loose cylinder and fastened with paper clips top and bottom. The cylinder then just stands in the bottom of the container.



**Figure 1:** Paper Chromatography set up (Courtesy Reference 1)

The reason for covering the container is to make sure that the atmosphere in the beaker is saturated with solvent vapor. Saturating with the atmosphere in the beaker with vapor stops the solvent from evaporating as it rises up the paper. As the solvent slowly travels up the paper, the different components of the mixture (see the spots on the line) travel at different rates and the mixture is separated into different spots.

Figure 1 shows what the plate might look like after the solvent has moved almost to the top. Further it

shows that the mixture consists of several components.

Similar to the previous article on Planer chromatography, there are several modes of paper chromatography. They are briefly explained here and the interested reader may refer for further details in literature.

### Ascending chromatography

As the name indicates, the solvent front moves upwards or simply ascends. The careful reader would find out in fact this happens against the gravity which is called capillary action. The solvent required for the development of the chromatogram supplied through the reservoir located at the bottom of beaker. In ascending technique the chromatogram is attached in a way that the spot is touched with the solvent where the solvent is at the bottom. The filter paper is attached to the tank by the paper support and filter paper will touch the solvent. However, the spot should not touch the solvent. In this method, the most polar substance will be at the bottom with respect to the tank whereas the least polar will be on the top end of the tank. Ascending technique is relatively a slow process as it operates against the gravity. A generic setup of such a system is shown in Figure 2.

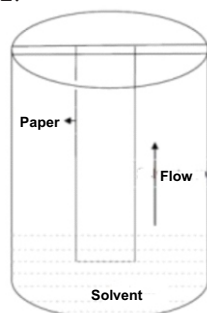


Figure 2: Ascending chromatography

### Descending chromatography

Contradictory to ascending chromatography, in this method the development of the paper occurs due to the solvent travel downwards on the paper where the separation occurs. Therefore, the solvent reservoir is located at the top. The movement of solvent is assisted by gravity besides the capillary action. As seen in Figure 3, the descending chromatography technique and setup are complex setup. Hence, this is built and can be purchased. The filter paper is attached to a paper support and is saturated with the stationary phase before it is hung. In this technique most polar substance will be on the top with respect to the tank whereas the least polar ones will be at the bottom. A generic setup of such a system is shown in Figure 3.

### Circular Chromatography

Here the solvent travels from center (mid-point)

towards periphery of circular chromatography paper. The entire system is kept in covered with a suitable lid (petridish) for the development of chromatogram. The wick at the center of paper dips into mobile phase in a petridish by which the solvent drains on to the paper and moves the sample radially to form the sample spots of different compounds as concentric rings. A generic setup of such a system is shown in Figure 4.

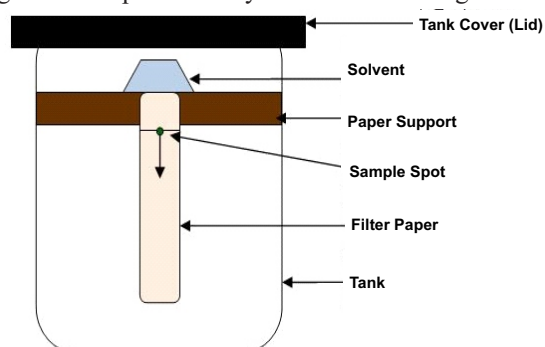


Figure 3: Descending chromatography

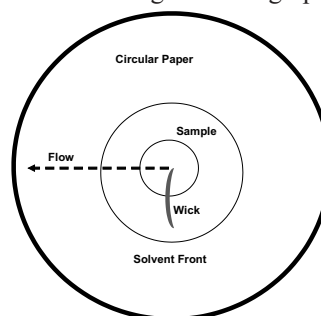


Figure 4: Circular Chromatography

### Two dimensional chromatography

Two dimensional technique is another complex set up which is used to separate complex mixtures. Here the chromatogram development occurs in two directions at right angles. The samples are spotted to one corner of rectangular paper and then allowed for first development with the solvent 1 as shown in Figure 5(a). Once that development is complete (Figure 5b), it may take sometimes several hours, the paper is again immersed in the mobile phase (this may or may not be the same solvent) at right angle to previous development for second chromatogram as shown in Figure 5c. The arrows in Figure 5b and 5c show the direction of development. It is interested to note that the development is always one directional. Although this will take some time, this allows a high degree of separation.

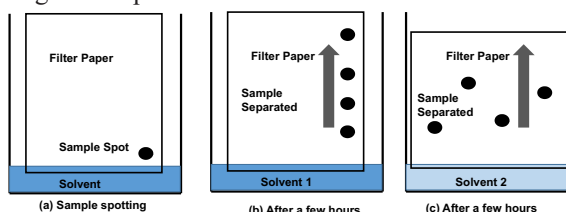


Figure 5: steps in 2D paper chromatography

Important things to note in paper chromatography;

- a) Selection of suitable type of development  
As discussed above selection of the correct type is very important. This depends on complexity of the mixture, solvent, paper etc.
- b) Selection of suitable filter paper  
Filter paper is selected based on pore size, quality of the sample to be separated, and also mode of development.
- c) Preparation of sample  
Preparation of sample involves dissolution of sample in suitable solvent used in making mobile phase. The solvent used should be inert with the sample under analysis.
- d) Spotting of sample on the paper  
Samples are to be spotted at proper position on the paper using preferably a capillary tube.
- e) Development of chromatogram  
Sample spotted paper is subjected to development by immersing it in the mobile phase. The mobile phase moves over the sample on the paper under the capillary action of paper or with gravity.
- f) Drying of the paper and detection of the compounds  
Once the development of chromatogram is over,

the paper is held carefully and dried using an air drier. Sometimes the detecting solution is sprayed in the developed paper and dried to identify the sample chromatogram spots.

#### Uses and applications of paper chromatography

Unlike other chromatographic techniques, paper chromatography is exclusively used for separation of mixtures having polar and nonpolar compounds. Some of its applications are listed as follows:

1. To separate of amino acids
2. To determine organic compounds
3. In pharma sector for determination of hormones, drugs, etc.
4. To evaluate inorganic compounds like salts and complexes

#### References

1. <http://www.chemguide.co.uk/analysis/chromatography/paper.html> (accessed April 28, 2015)
2. <http://genchem.rutgers.edu/chrompap.html> (accessed April 28, 2015)
3. Harvey, D. *Modern Analytical Chemistry*, The McGraw-Hill Companies Inc., 2000

### New low-calorie rice could help cut rising obesity rates



**Mr. Sudhair James**

Mr. Sudhair James, who graduated from the College of Chemical Sciences in 2014, presented his undergraduate research work at the 249<sup>th</sup> National Meeting & Exposition of the American Chemical Society (ACS) which was held in Denver, Colorado, USA from March 22-26, 2015. The project was supervised by Professor Pushparajah Thavarajah (USA). Other supervisors were Dr. S. Premakumara and Dr. W. K. S. M. Abeysekera from the Industrial Technology Institute, Professor S. Sotheeswaran from the College of Chemical Sciences and Dr. D. Pushparajah (USA). Mr. James and the team discovered that increasing the resistant starch concentration of the rice by using a simple cooking method, reduces the calorie content of the rice. In this study they had tested 38 rice varieties of Sri Lanka. According to the developed method, a small amount of coconut oil (One teaspoon

of oil for a half a cup) has to be added to the boiling water. The dry rice is added cooked for about 40 minutes. After cooking, the rice has to be refrigerated for about 12 hours. They have found the reduction of number of calories by 10-15%.

The success story of this research was written by over 50+ countries worldwide and over 1000+ articles have been written to date. Top media: BBC English, Tamil and Sinhala, Yahoo news and Washington post also have covered this story.

This project was mainly funded by the College of Chemical Sciences, Institute of Chemistry Ceylon and Prof. Thavarajah's US and SL research programmes.

We congratulate Mr. Sudhair James and his team on this achievement and wish them all the best for their future research work. We also thank Mr. James for the recognition given to the College of Chemical Sciences, Institute of Chemistry Ceylon.

## CCS Analytical and Consultancy Services

We are happy to announce that the following services will be provided by the College of Chemical Sciences (CCS), the educational arm of **Institute of Chemistry Ceylon**.

**The H D Gunawardhana Instruments Center of the College is equipped with the following advanced instruments**

Gas Chromatograph  
(GL sciences 4000, Japan)



Atomic Absorption Spectrometer with flame  
and Graphite furnace  
(Hitachi ZA 3000)



Fluorescence Spectrophotometer  
(Hitachi, F 2700)



FT-IR spectrophotometer  
(ABB MB 3000)



UV- Visible Spectrophotometer  
(Hitachi U 2910 )



TOTP- H 50 ml High Temperature-  
High Pressure Reactor

### For the Industry

- Consultancy Services
- Method Development
- R&D services

### Analytical Services Offered

- Water Quality Parameters (DOD, BOD, COD, pH, Conductivity, Hardness, turbidity, Nitrate, Nitrite, and Total Nitrogen etc).
- Food and Nutrient Analysis.
- Analysis of specific chemicals in various samples.
- Analysis of heavy metals
- Analysis of Paint, textile dyes, pigments etc

### Contact any of the following officials for your requirements of Analytical/ Consultancy Services

The Management Committee on Analytical and Consultancy Services

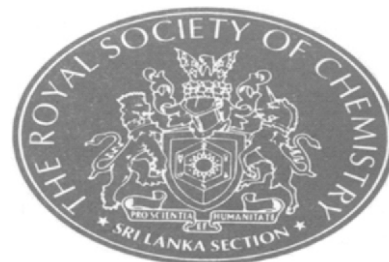
**Prof. MDP De Costa**, Senior Professor, (Academic laboratory and Analytical / Consultancy Services Coordinator)

**Dr. USK Welivegamage**, Senior Lecturer

**Dr. C Udawatte**, Senior Lecturer

**Dr. R Parthipan**, Senior Lecturer

Tel: 011 2861231, 2861653, 4615230



# RSC NEWS

## THE ROYAL SOCIETY OF CHEMISTRY SRI LANKA SECTION

### 1. Membership

According to the records sent to us from the parent body, a breakdown of the membership is as follows:-

| Category                                | Number    |
|-----------------------------------------|-----------|
| CChem, FRSC                             | 11        |
| FRSC                                    | 05        |
| CChem, MRSC                             | 10        |
| MRSC                                    | 20        |
| AMRSC                                   | 08        |
| Affiliate /Under Graduate.              | 06        |
| <b>Total Membership as at July 2014</b> | <b>60</b> |

### 2. Committee of Management

The following were elected to the Committee at the 53<sup>rd</sup> Annual General Meeting held in July 2014.

|                 |                           |
|-----------------|---------------------------|
| Chairman        | - Mr. R M G B Rajanayake  |
| Vice Chairman   | - Prof. Sudantha Liyanage |
| Chairman Elect  | - Mr. I M S Herath        |
| Hony. Secretary | - Mr. Sulith Liyanage     |
| Hony. Treasurer | - Mr. I M S Herath        |

Committee Members -

Prof. W S Fernando  
Dr. M P Deeyamulla  
Mr. T M Kumar  
Mr. W K Samarakoon  
Mr. S Perasiriyan  
Mr. W J P D Jayalath

### 3. Activities

3.1 Contributions to Activities of the Institute of Chemistry Ceylon

(a) Full page advertisement of "Chemistry in Sri Lanka".

(b) Contribution for the Interschool Chemistry Quiz

(c) Award for the Best Performance at the Graduate ship Examination in Chemistry Part II Theory Examination

3.2 All - Island Inter School Chemistry Essay Competition.

3.3 Inter - University Chemistry Competition.

3.4 A/L teacher workshops.

3.5 Advanced Level chemistry seminar.

3.6 Book donation programmes

3.7 Industrial Visit.

3.8 Collaborations with SLAAS -E2 Work Shop and Seminars

3.9 Supporting Chemical Societies of Universities of Sri Lanka

### 4. Web Site

The members are reminded of the web site of our Section, the address of which is as follows:-  
[www.rsc.org/Membership/Networking/InternationalSections/SriLanka/index.asp](http://www.rsc.org/Membership/Networking/InternationalSections/SriLanka/index.asp).

Mr. Sulith Liyanage  
*Hony Secretary*

## PUBLICATIONS OF THE INSTITUTE OF CHEMISTRY CEYLON

| Monograph                  | Title                                                         | Author                                      | Price             |    |
|----------------------------|---------------------------------------------------------------|---------------------------------------------|-------------------|----|
| 01                         | Textile Fibers                                                | Mr T Rajasekeram                            | Rs.50/-           |    |
| 02                         | Principles of Food Preservation                               | Prof U Samarajeewa                          | Rs.75/-           | 03 |
| Biotechnology              |                                                               | Prof C P D W Mathew                         | Rs.75/-           | 04 |
| Recombinant DNA Technology |                                                               | Prof J Welihinda                            | Rs.75/-           |    |
| 05                         | *Natural Toxins in Foodstuffs                                 | Prof E R Jansz & Ms A S Perera              | Rs.50/-           |    |
| 06                         | Fat Soluble Vitamins                                          | Prof E R Jansz & Ms S Malavidana            | Rs.50/-           |    |
| 07                         | Nucleic Acid and Protein Synthesis                            | Prof J Welihinda                            | Rs.75/-           |    |
| 08                         | Extraction of Energy from Food                                | Prof J Welihinda                            | Rs.50/-           |    |
| 09                         | Corrosion of Materials                                        | Dr A M M Amirudeen                          | Rs.75/-           |    |
| 10                         | Vitamin C-Have all its mysteries                              | Prof E R Jansz & Ms S T C Mahavithanage     | Rs.75/-           |    |
| 11                         | *Environmental Organic Chemistry (second edition)             | Prof S Sotheeswaran                         | Rs.150/- (US \$3) |    |
| 12                         | Enzyme Kinetics and Catalysis                                 | Prof (Mrs) S A Deraniyagala                 | Rs.100/-          |    |
| 13                         | Insecticides                                                  | Prof (Mrs) Sukumal Wimalasena               | Rs.95/-           |    |
| 14                         | Organotransition Metal Catalysts                              | Prof S P Deraniyagala & Prof M D P De Costa | Rs.75/-           |    |
| 15                         | Some Important Aspects of Polymer Characterization            | Prof L Karunanayake                         | Rs.75/-           |    |
| 16                         | Hard & Soft Acids & Bases                                     | Prof (Mrs) Janitha A Liyanage               | Rs.65/-           |    |
| 17                         | Chemistry of Metallocenes                                     | Prof Sarath D Perera                        | Rs.65/-           |    |
| 18                         | Lasers                                                        | Prof P P M Jayaweera                        | Rs.65/-           |    |
| 19                         | *Life and Metals                                              | Prof (Mrs) Janitha A Liyanage               | Rs.75/-           |    |
| 21                         | *Silicones                                                    | Prof Sudantha Liyanage                      | Rs.65/-           |    |
| 22                         | Pericyclic Reactions: Theory and Applications                 | Dr M D P De Costa                           | Rs.65/-           |    |
| 23                         | Inorganic NMR Spectroscopy                                    | Prof K S D Perera                           | Rs.65/-           |    |
| 24                         | Industrial Polymers                                           | Prof L Karunanayake                         | Rs.75/-           |    |
| 25                         | *NMR Spectroscopy                                             | Dr (Mrs) D T U Abeytunga                    | Rs.65/-           |    |
| 26                         | Mosquito Coils and Consumer                                   | Ms D K Galpoththage                         | Rs.100/-          |    |
| 27                         | *Atomic Absorption Spectrometry                               | Prof K A S Pathiratne                       | Rs.100/-          |    |
| 28                         | Iron Management on Biological Systems                         | Prof (Ms) R D Wijesekera                    | Rs.100/-          |    |
| 29                         | Nutritional Antioxidants                                      | Prof. (Mrs) Sukumal Wimalasena              | Rs.100/-          |    |
| 30                         | *f-Block Elements                                             | Prof Sudantha Liyanage                      | Rs.65/-           |    |
| 31                         | Scientific Measurements and Calculations                      | Prof (Mrs) S A Deraniyagala                 | Rs. 80/-          |    |
| 32                         | Applications of Organometallic compounds in Organic Synthesis | Dr. Chayanika Padumadasa                    | Rs. 60/-          |    |
| 32                         | Organosulfur Compounds in Nature                              | Prof. S Sotheeswaran                        | Rs. 200/-         |    |

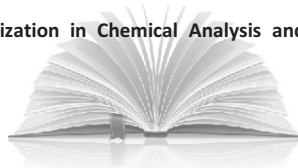
\* - Second Edition /new print published on popular demand

### CCS Publications

|    |                                                |                                                 |           |
|----|------------------------------------------------|-------------------------------------------------|-----------|
| 01 | Functional Group Analysis in Organic Chemistry | Prof A A L Gunatilake                           | Rs. 175/- |
| 02 | Zinc Metalloproteins                           | Prof S Sotheeswaran<br>Prof (Ms) R D Wijesekera | Rs. 175/- |

### General Publications

- ★ **Chemist & The Environment** (Rs.300/-)
- ★ **Infrastructure Support Services for Industrial Development** (Rs.200/-)
- ★ **Chemical Industries in Sri Lanka – Part II** (Members: Rs. 200/-, Non-members: Rs.275/-)
- ★ **Proceedings of the Workshop on the Technological aspects of the Production & Processing of Essential oils in Sri Lanka** (Rs.100/-)
- ★ **Proceedings of the Training Seminar on Towards a Cleaner Industrial Environment in the New Millennium** (Rs.150/-)
- ★ **A-Level Chemistry Facts, Patterns & Principles by Dr. Seetha I Rodrigo** (Rs.1500/-)
- ★ **Proceedings of the Prof R S Ramakrishna Memorial Training Seminar on Modern Analytical Methods**(Rs.200/-)
- ★ **Historical Accounts of the Educational Activities (1972 - 2004)** (Rs.350/-)
- ★ **Proceedings of the Training Seminar cum Workshop on Sampling, Statistics and Standardization in Chemical Analysis and Environmental Management** (Rs.150/-)
- ★ **Polymer Industries of Sri Lanka** (Rs. 200/-)
- ★ **Industry & Environment** (Rs. 200/-)
- ★ **Herbal Medicine Phytopharmaceuticals and Other Natural Products: Trends and Advances** (Rs. 500/-)
- ★ **Chemistry in Sri Lanka** (Rs. 150/-)



# Eleventh Convocation held at the Eagle's Lakeside on 19<sup>th</sup> February 2015. 106 Graduate Chemists and 80 DLTC Diplomates Pass Out

