Amylose content of some Sri Lankan rice varieties grown in Yala and Maha seasons

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Rice (*Oryza sativa* L.) is the most important cereal crop in the developing world and is the staple food for over half the world's population including Sri Lanka. Per capita consumption of rice in Sri Lanka is reported as 100 kg/ year/ person and it remains the major source of calories (45%) for Sri Lankans.[1]

Rice mainly consists of starch. Rice starch contains two major chemical constituents amylose and amylopectin, which govern much of the sensory and cooking characteristics of rice. In most varieties of rice the amylose content varies from 12-35% of the total starch. The non sticky types of rice preferred by Sri Lankans contain 25-30% amylose.[2]

Since there is a growing interest on developing various bakery and confectionary products from rice flour it is important to understand the contents of these two important chemicals in Sri Lankan rice varieties. Since the properties of rice in cooking and sensory characters depend on amylose and amylopectin, the amylose content in thirty different varieties of rice (both traditional and improved) consumed by Sri Lankans obtained from the Regional Rice Research and Development Center (RRRDC) Bombuwala were studied. Samples analyzed were from Yala (2006) and Maha (2006-2007).

Amylose content (AC) in rice was determined by the standard Iodine Colorimetric Method of Juliano, 1985.[3] AC of the 30 rice varieties tested is given in Table 1. There was a significant difference in the AC between the rice varieties ($P<0.05$). However, no significant difference was observed in the AC of the rice due to seasonal variation.

Table 1: Amylose content of some Sri Lankan traditional and improved rice varieties

Variety	Pericarp Color	Maha Season (%)	Yala season (%)	Classification
*Herath Banda ^{bcdef}	Red	27.51± 0.11	28.50 ± 0.32	High
*Kalu Heeneti ^{ab}	Red	29.15 ± 0.08	29.20 ± 0.26	High
*Rathu Heeneti ^{g}	Red	26.59 ± 0.13	26.42 ± 0.25	High
*Sudu Heeneti ^{efg}	Red	27.53 ± 0.17	27.17 ± 0.13	High
*Beath Heeneti ^{efg}	Red	28.11 ± 0.25	26.78 ± 0.64	High
*Goda Heeneti ^{bcdef}	Red	27.31 ± 0.21	29.01 ± 0.29	High
*Rathal ^{a}	White	29.27 ± 0.94	30.05 ± 0.48	High
*Batapolal ^{abcdef}	Red	28.64 ± 0.20	28.11 ± 0.29	High
*Kahata Wee ^{ab}	Red	30.94 ± 0.39	27.60 ± 0.21	High
*Dik Wee ^{ab}	Red	28.28 ± 0.29	30.07 ± 0.29	High
*Kalubala Wee ^{abcde}	Red	29.03 ± 0.41	28.23 ± 0.70	High
*Molligoda ^{bcdef}	Red	28.79 ± 0.44	27.24 ± 0.17	High
*Kottayar ^{abcdef}	Red	27.63 ± 0.21	28.96 ± 0.35	High
*Dahanala ^{defg}	Red	27.14 ± 0.20	27.99 ± 0.40	High
*Wanni Dahanala ^{cdefg}	Red	28.52 ± 0.33	26.83 ± 0.26	High
*Pachchaperumal ^{cdefg}	Red	27.65 ± 0.38	27.65 ± 0.13	High
*Sulai ^{abcde}	Red	28.79 ± 0.24	28.64 ± 0.20	High
*Kattamanjal ^{efg}	White	26.22 ± 0.52	28.43 ± 0.26	High
*Madathawalu ^{abcdef}	Red	28.60 ± 0.20	28.18 ± 0.33	High
*Rath Suwadal ^{ab}	Red	29.10 ± 0.23	29.20 ± 0.67	High
*Hondarawala ^{abcd}	Red	29.20 ± 0.26	28.69 ± 0.51	High
*Masuran ^{bcdef}	Red	27.82 ± 0.21	28.60 ± 0.48	High
*Gonabaru ^{bcdef}	Red	30.19 ± 0.25	27.17 ± 0.39	High
Suduru Samba ^{h}	White	24.21 ± 0.21	24.70 ± 0.18	Intermediate
Bw 267 -3 ^{abcde}	White	28.28 ± 0.37	29.08 ± 0.50	High
Bw 272 - 6b ^{fg}	Red	26.68 ± 0.20	27.58 ± 0.25	High
Bg 352 ^{ab}	White	28.98 ± 0.54	29.90 ± 0.17	High
Bg 406 ^{h}	Red	25.16 ± 0.27	25.01 ± 0.43	High
At 353 ^{bcdef}	Red	28.47 ± 0.25	27.63 ± 0.20	High
At 354 ^{abc}	White	28.23 ± 0.36	29.95 ± 0.17	High

Data represented as mean ± SEM. Varieties with the same letter are not significantly different.

* = Traditional varieties

Further, results indicated that the amylose content of selected Sri Lankan rice varieties varies from 24-31%. All the traditional varieties had high amylose contents (26-31%) and Suduru samba, which is a hybrid variety had intermediate amylose content both in Yala (24.7 ± 0.2) and Maha (24.2 ± 0.2) seasons.

Since high amylose content is associated with the lower glycemic and insulin responses these results together with the further findings on factors affecting the glycemic index of rice would be useful in breeding programs in development of rice varieties with acceptable grain quality with low glycemic indices and functional properties.

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Preparation of *Pinnatu* destroys the hypocholesterolaemic effect of palmyrah fruit pulp

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Palmyrah Fruit Pulp (PFP) is extensively used in many food preparations and it accounts for a considerable portion of the versatility in use of the palmyrah palm. Past studies have reported the hypocholesterolaemic effect of PFP. The Soluble Dietary Fibre (SDF) which is dominated by high M.W. pectin has been suggested as the causative agent. The present study was undertaken to determine if *pinnatu* (dried PFP, fruit leather) possesses hypocholesterolaemic activity and if so, to study whether the effect is due to pectin.

Pinnatu (prepared by drying PFP in layers in a Mitchel air oven at 65-67 ÚC) contained 6.6% SDF, 23.1% on dry weight and 92% of SDF comprised pectin. However neither standard diet containing *pinnatu* [11% *pinnatu* (in place of 11% maize) incorporated WHO standard diet] nor pectin extracted from *pinnatu* (incorporated at a double dose to the above experiment, in place of grass powder) could indicate any hypocholesterolaemic effect on male Wistar rats.

Pinnatu pectin on separation on Sepharose gel chromatography showed the typical polydisperse pattern with a shift to the high elution volume pattern indicating the hydrolysis of pectin while processing. It was also found that *pinnatu* has exopectinase activity. Thus *pinnatu* was prepared using a modified method in order to minimize the hydrolysis of high molecular weight pectin. This was achieved by bringing down the pH of PFP (soon after collection) to 2.5 using HCl and heating at 70-80ÚC for 15 min. After heating the pH was readjusted (to 4.6) and thus treated PFP was used to prepare *pinnatu*. The Sepharose gel chromatography pattern of the isolated pectin of the modified *pinnatu* showed no degradation of pectin. Therefore it was evident that the inherent pectinase activity had been successfully inhibited. For logistic reasons *pinnatu* had to be kept in the freezer for 6-7 months. On testing this stored *pinnatu* pectin it was found that there was a slight shift to the lower elution volume indicating a slight hydrolysis. Feeding Wistar rats with this *pinnatu* showed no hypocholesterolaemic effect ($p=0.85$) indicating that the hypocholesterolaemic effect of PFP is destroyed during the preparation and storage of *pinnatu*.

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Effects of *Gymnema lactiferum* leaf on serum glucose and cholesterol levels of normo-glycaemic and streptozotocin induced diabetic rats

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Gymnema lactiferum var. lactiferum is a plant that closely resembles *Gymnema sylvestre* which possesses a proven hypoglycaemic activity. The present study evaluates the effects of *Gymnema lactiferum* on normo-glycaemic and type 2 diabetic rats with respect to their blood glucose and cholesterol levels.

Male Wistar rats bred at MRI (8-10 weeks old; n=6 in each group) were used in the normo-glycaemic study. Animals of the test group were fed with *Gymnema lactiferum* leaf powder incorporated WHO standard diet on a dose of 0.2 g/Kg body weight/day/ rat for a period of four weeks. The leaf powder was substituted for grass powder in the WHO standard diet to maintain the calorie and fibre content. The fasting blood glucose (FBS) and the serum total cholesterol levels were determined on day zero and after four

weeks. A glucose challenge was also performed on the last day of the experiment. Results did not indicate any significant reduction in FBS (p=0.64) or serum total cholesterol levels (p=0.49) in normo-glycaemic Wistar rats. The result of the glucose challenge was also insignificant (p=0.26).

The next study was carried out to study the effect of a water extract of *Gymnema lactiferum* leaf powder (prepared by heating the leaf powder at 60ÚC for 6 hours followed by filtering and freeze-drying the extract) on glucose challenge of normo-glycaemic Wistar rats and the result was not significant (p=0.09) at a dose of 0.2 g/Kg body weight/rat.

Male Long-Evans rats (150-210 g) bred at BIRDEM animal house, Bangladesh were used for the diabetic studies. Animals were made diabetic by a single intraperitoneal injection of streptozotocin to 48 h old pups and experiments were carried out after three months. The test group was fed with *Gymnema lactiferum* leaf powder at a dose of 1.25 g/Kg body weight/day once daily for 28 consecutive days. A Standard drug group was treated with glibenclamide (5 mg/Kg body weight). A water control group was also included in the study. In diabetic rats any acute effect on blood glucose levels after a glucose challenge was not observed on feeding with *Gymnema lactiferum*. In the chronic study it was observed that the body weights of rats increased (not significantly) in all groups. Therefore it can be concluded that *Gymnema lactiferum* does not have any effect on degradation of depots of fat and it can maintain the body weight in type 2 diabetic state. There was a gradual reduction in FBS of type 2 diabetic rats and the reduction was significant (p=0.001) after 28 days in comparison to the day zero values of the treated group. Similarly, a significant (p=0.015) decline could be seen in the serum total cholesterol levels. However the serum HDL-cholesterol (p=0.165), LDL-cholesterol (p=0.189) and TG (0.465) levels were not affected significantly. The unchanged serum ALT (p=0.261) and creatinine (p=0.797) levels indicated that the consumption of *Gymnema lactiferum* leaf powder over a period of time did not cause any detrimental effects on type 2 diabetic rats. It is concluded that *Gymnema lactiferum* leaf possesses beneficial effects in type 2 diabetic model rats with respect to their glycaemic and lipidemic status.

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Effects of *Gymnema lactiferum* on glycaemic and lipidemic status in type 2 diabetics

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Gymnema lactiferum var. lactiferum (Sinhala – Kuringnan) has a long folkloric history in the treatment of diabetes. The present study evaluates the effect of *G. lactiferum* leaf powder on glycaemic and lipidemic status of type 2 diabetics. Such a study has not been conducted before. Type 2 diabetic patients (n=12, age between 30-60 yrs) were recruited from the Out-Patient Department of Bangladesh Institute for Research and Rehabilitation in Diabetes Endocrine and Metabolic Disorders (BIRDEM), Dhaka. Patients having hypercholesterolaemia, who were not taking any lipid lowering drug and not suffering from any recurrent illnesses or any other endocrinological diseases were included in the study. Another group of type 2 diabetic and hypercholesterolaemic patients (n=14) served as the control group. The chronic consumption of the leaf powder (at a dose of 3.5 g, twice a day) for a period of four weeks resulted in a gradual reduction of fasting blood sugar levels and on day 29 the reduction became significant (p=0.002, 18.15% reduction). Both treated and control groups of patients were on their conventional oral hypoglycaemic drug doses. Therefore the results show the added advantage of the consumption of *G. lactiferum* leaf powder to their glycaemic control. Although any acute glucose lowering effect could not be seen, as judged by the post-prandial blood glucose levels on day zero, the chronic consumption of the leaf powder reduced the post-prandial blood glucose levels significantly (p=0.026 for 60 min and p=0.022 for 120 min) on day 29. A significant decline (p=0.012) was observed in the HbA_{IC} levels at the end of the study despite the short (four weeks) duration of time. Serum total cholesterol and LDL levels of the treated group were reduced significantly (p=0.004 with a 12.3% reduction and p=0.023 with a 15.5% reduction respectively). The leaf powder therapy indicates no pronounced effect on serum HDL levels but there was a 7.0 % reduction in serum TG levels. Serum ALT and creatinine levels remained unchanged throughout the study period in both test and control groups. No complaints were made by the patients on any undesirable effects indicating that there were no detrimental effects due to chronic consumption of leaf powder. There was a 0.74% reduction in the body weight of the treated group which was insignificant. From these results it can be concluded that the *G. lactiferum* leaf powder improves the glycaemic and lipidemic status in type 2 diabetes and it could be further postulated that the leaf would make a good functional food.

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The cytotoxic and hyper haemolytic steroidal saponins of palmyrah flour

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During the course of bioactivity directed separation for the reported neurotoxic principle of palmyrah flour (PF) a saponin was extracted from the Normal Phase Medium Pressure Liquid Chromatography (MPLC) gradient of 100% methanol. This was crystallized from a MeOH:EtOAc mixture. This compound resulted in cytotoxic activity to melanoma cells at 100 µg/mL. Subjecting the compound to ^{13}C NMR it was found to be clearly related in structure to spirostane tetraglycoside. However the links between glycosides are more likely to be 1, 2 and 1, 6 instead of 1, 2 and 1, 4 when compared with published data.

A similar bioactivity directed separation of the mosquito larvicidal saponin fraction of palmyrah flour with normal phase MPLC above 90% MeOH:EtOAc mixture and the reverse phase open column yielded the saponin that was crystallized from a methanol ethyl acetate mixture under low temperature. The resulting crystals were off-white in colour. This compound showed high haemolysis on human red blood cells compared to other saponins of palmyrah. The compound was subjected to microTOF mass analysis M^+/z peak at 1534 (M + H) and the following peaks 1307 (M + H –$C_{16}H_{20}O$), 1161 (M + H –$C_{16}H_{20}O$ – Rha), 1015 (M + H –$C_{16}H_{20}O$ – 2 Rha), 869 (M + H –$C_{16}H_{20}O$ – 3 Rha), 732 (M + H –$C_{16}H_{20}O$ – 4 Rha), 577 (M + H –$C_{16}H_{20}O$ – 5 Rha), 415, (M + H –$C_{16}H_{20}O$ – 5 Rha - Glc) were obtained. The steroid molecule having a MW of 414 could be spirostane or â-sitosterol. Studies show the presence of 5 rhanmosyl and 1 glucosyl residue and a fragment of 228. For the compound to be hyper haemolytic this fragment must be hydrophobic. Fatty acid derivatives of saponins have been reported before in PF and other plants. However the molecular weight does not compare with that of a common fatty acid.

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In-vitro assay of hydrolyzed casein and casein fractions isolated from milk and their inhibitory effect on Angiotensin converting enzyme activity

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Milk proteins mainly the caseins have different *in-vitro* and *in-vivo* biological values. These proteins serve as the precursor of different peptides possessing various biological activities such as anti-hypertensivity, anti-thrombotic immunomodulation, enzyme inhibition, and digestive functioning. Caseino phosphopeptides are a group of peptides derived from the enzymatic hydrolysis of casein which has a wide variety of health benefits. Therefore, a study was carried out to determine the effect of predigested casein and fractions of casein on Angiotensin Converting Enzyme (ACE). ACE plays a major role in regulating the blood pressure in humans and animals. This enzyme catalyses the conversion of angiotensin I to angiotensin II which in turn plays a major role in regulating the blood pressure, blood volume and water retention. Casein contributes to the major protein fraction (80%) of milk and consist of α-casein, β-casein, and κ-casein. These proteins were precipitated at their iso-electric pH from skim milk which was obtained by removing the fat by centrifuging at 10,000 rpm at 4 °C for 30 minutes. These precipitates were run in SDS PAGE to obtain a single band separation to confirm a good purified separation. Each separated sample was subjected to sequential enzymatic digestion by adding pepsin at pH 1.5 and then incubating at 37 °C for 24 h in a shaking water bath. The pH was adjusted to 7.8 and the sample was digested with a mixture of pancreatin and trypsin for 24 h at 37 °C. The samples were mixed and the pH was monitored from time to time and readjusted to obtain the optimum pH for the respective enzyme. The samples were centrifuged and the clear supernatant obtained was used for the assay. The ACE inhibitory activity was measured using some modification made to the original method of Cushman and Cheung. The extent of inhibition by the peptides formed after digestion was then calculated. The substrate free buffer was considered to have 100% activity and the blank as 0% activity. Trypsin was used in addition to pancreatin as literature indicates that most of the bioactive peptides are produced using pepsin and trypsin.

The highest ACE inhibiting activity of 83.6% was obtained with total casein followed by 82.1% inhibition with β-casein, 80.1% by κ-casein, and 43.2 % with α-casein.

These results indicate that casein inhibits ACE activity and hence may be of value in the dietary management of high blood pressure in hypertensive

subjects. Since no studies have been reported in human subjects it may be necessary to carry out studies on human subjects before any dietary advice is given.

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Antioxidant properties of unconventional leafy vegetables commonly consumed by Sri Lankans

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The present study reports on the antioxidant properties (content of total phenols, flavonoids, reducing power, DPPH radical scavenging activity) of three varieties of unconventional leafy vegetables consumed by Sri Lankans namely *Pisonia grandis* (Sinh. Wathabanga), *Cassia occidentalis* (Sinh. Penithora) and *Asterachantha longifolia* (Sinh. Neeramulliya). These leafy vegetables are commonly consumed by rural people as cooked salads (Sinh. Mallum) in Sri Lanka and are reported to have medicinal properties.

The content of total phenols and free phenols were determined for refluxed aqueous and methanol extracts using Folin Ciocalteu reagent. The free flavonoid content in refluxed aqueous and methanol extracts were determined using $AlCl_3$. The reducing power and free radical scavenging power were determined using potassium ferricyanide and DPPH radical respectively. Each determination was carried out in six replicates and for each determination pooled out leaves from three bundles were used. The results were subjected to statistical analysis.

The present study revealed that the methanol extract {6.5 (*C. occidentalis*) - 3.7 (*A. longifolia*) mg g^{-1}} contained more free phenols than the aqueous extract {2.4 (*A. longifolia*) - 1.7 (*P. grandis*) mg g^{-1}} while total phenol was higher in the water extracts {7.4 (*P. grandis*) – 3.6 (*C. occidentalis*) mg g^{-1}} than in the methanol extracts {3.9 (*P. grandis*) - 3.1(*C. occidentalis*) mg g^{-1}} indicating that the content of water soluble phenols in conjugated form is high. The methanol soluble and water soluble free phenols were highest in *C. occidentalis* (6.5 ± 0.1 mg g^{-1}) and *A. longifolia* (2.4 ±0.2 mg g^{-1}) respectively. For the aqueous (7.4 ±0.1 mg g^{-1}) and methanol extracts (3.9 ±0.3 mg g^{-1}) the total phenol content was highest in *P. grandis*. Further, this study revealed that most of the phenols in *P. grandis* are conjugated. The free phenol content in the methanol extract has decreased compared to the aqueous extract. This may be due to the phenols undergoing reactions or decomposing during heating with acidic methanol. The flavonoid content was higher in the methanol extracts {3.5 (*P. grandis*) - 0.6 (*C. occidentalis*) mg g^{-1}} than in the aqueous extracts 1.1 (*P. grandis*) - 0.5 (*A. longifolia*) mg g^{-1}} for all the leafy vegetables analyzed.

Reducing power increased with the concentration of the leafy vegetables for both methanol and aqueous extracts. The highest reducing power for the aqueous and methanol extracts was obtained in *A. longifolia* and C. *occidentalis* respectively. The methanol extract which had a higher free phenol & flavonoid content than the aqueous extract had a higher reducing power. But the reducing power measured using $K_4Fe(CN)_6$ and antioxidant power of aqueous and methanol extracts of the leaves analyzed did not show a direct relationship to the phenol and flavonoid content.

DPPH radical scavenging power as determined by EC_{50} was highest in *P. grandis* and lowest in *C. occidentalis*. The flavonoid content had a direct relation to the DPPH radical scavenging power of both methanol and water extracts but the phenol content did not show a direct relation.

The results of the present study indicate that of the unconconventional leaves analysed *P. grandis* is the best sources of antioxidants while *C. occidentalis* is the poorest and that there are compounds other than phenols and flavonoids that could contribute to the reducing power and antioxidant properties of the leaves analyzed. Further, synergistic effects of these compounds may also contribute to antioxidant and reducing power.

Effect of pretreatments on extending the shelf life of minimally processed rhizomes of *Lasia spinosa*

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The rhizomes of *Lasia spinosa* (Sinh. Kohila) is a vegetable of medicinal value consumed by Sri Lankans. It also has a high fibre content. Due to a busy life style, most of the present day Sri Lankan house wives are reluctant to cook *L. spinosa* as cleaning prior to cooking is difficult and it cannot be cleaned and kept for a long time without undergoing browning. If minimally processed *L. spinosa* is available in a 'ready to cook' form it will be a good marketable product and will become a popular vegetable among the urban population. The present study was undertaken to deduce the optimal conditions required to produce minimally processed *L. spinosa* using the minimum amount of synthetic chemicals and to deduce the nutrient value of the product. Studies were carried out with two varieties of *L. spinosa* that are found in markets of Sri Lanka and are referred to as *L. spinosa* ver.1 ('big kohila') and *L. spinosa* ver.2 ('finger kohila').

Preliminary studies were carried out to deduce the best conditions for minimal processing of *L. spinosa* by packing pre-treated, cleaned cut chips of *L. spinosa* in low density polyethylene (150 gauge) pouches (6 x 6 inches) and storing at room temperature and 4 °C for seven days. *L. spinosa* chips were washed with distilled water and 100 ppm chlorinated water. For pre-treatments, dipping in distilled water (T_1) (control), 2% (w/v) calcium chloride (T_2), 0.5% (w/v) citric acid (T_3), 2% (w/v) calcium chloride + 0.5% (w/v) citric acid (T_4), 0.5% (w/v) ascorbic acid (T_5), 1% (w/v) lime juice (T_6), 0.5% (w/v) ascorbic acid + 1% (w/v) lime juice (T_7), 0.5% (w/v) ascorbic acid+0.5% (w/v) citric acid (T_8), 0.5% (w/v) sodium metabisulphite (T_9), 0.1% (w/v) acetic acid (T_{10}) and 0.1% (w/v) acetic acid+0.5% (w/v) ascorbic acid (T_{11}) were used. Based on the physical appearance, for samples stored at 4 °C for seven days, pretreatments T_1 (control), T_3, T_4, T_5 and T_8 were selected for the final experiment. It was observed that the selected pretreatments were better than the commonly used preservative sodium metabisulphite. pH, ascorbic acid content, texture and degree of browning were measured after the first, third, fifth and seventh day of storage while microbiological assays (aerobic plate, coliform, *E. coli* and *Salmonella*) were carried out after the seventh day. Based on the results of the microbiological studies, samples T_1, T_3 and T_4 were selected for sensory evaluation (colour, odour, texture, taste and overall acceptability) and determination of crude protein and crude fibre.

Microbiological assays and sensory evaluation of the cooked product (taste, texture, odour), consumer acceptability, economical feasibility, and head space analysis indicated that the best condition for minimally processed *L. spinosa* Ver.1 was T_3 although degree of browning, texture and nutrient content (proteins, crude fibre) gave better results with T_4. For Ver. 2 treating with 0.5% (w/v) citric acid was the best. Storing at room temperature was totally unsuccessful. For both samples T_3 and T_4 there was a decrease in the protein content and increase in fibre.

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Bioactivity directed separation of the neutotoxins of palmyrah flour and studies on its structure

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The neurotoxicity of palmyrah flour was known from 1971. Using a tadpole assay Greig and co-workers postulated a saponin with a tertiary amine as the toxic factor with an approximate MW of 1300. The objective of the present study was to isolate the neurotoxin/s by bioactivity directed separation using Wistar rats by intravenous injection. It is known that the toxin was extractable to ethanol or methanol. A crude extract of 12 g obtained from 500 g of palmyrah seed shoot was subjected to ion exchange chromatography, MPLC and preparative TLC respectively. It was found that the active fraction contained two major ninhydrin positive spots at R_f 0.65 and 0.72 values and a non-ninhydrin positive spot at R_f 0.69. Separation of individual compounds resulted in negative toxic effects at 100 – 172 mg/Kg body weight. However, a mixture of two ninhydrin positive spots at 63 mg/Kg body weight produced the toxic symptoms indicating synergism. Both these compounds were mildly fluorescent. The fluorescence was due to the impurity which was removed by Reverse Phase Recycle HPLC (ratio of the sample: impurity was approximately 18:1). Subjecting the pure compounds to ^{1}H-NMR and ^{13}C NMR showed a spirostane aglycone. The three sugars were two rhamnoses and another sugar which is postulated to be a glucosamine because the compound is ninhydrin positive. There were no interpretable differences in ^{1}H NMR of the other compound involved

in the synergistic effect, thus indicating a probable structural or conformational isomerism.

It is the first time that a neurotoxin of this class has been identified. These are steroidal saponins with a NH_2 substitute. It has been observed by a previous worker that palmyrah flour heated to 80°C for 45 min had lost neurotoxicity. The separated neurotoxic compounds given the same treatments showed no significant changes in ^1H NMR. A past study has indicated that the neurotoxic effect is due to rupture of the mitochondrial membrane of the liver. However the neurotoxic symptoms are more likely to arise if the mitochondria of the nervous system are affected. This could explain the neurotoxic symptoms.

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Isolation of intergric acid from the fungus *Xylaria hypoxylon*

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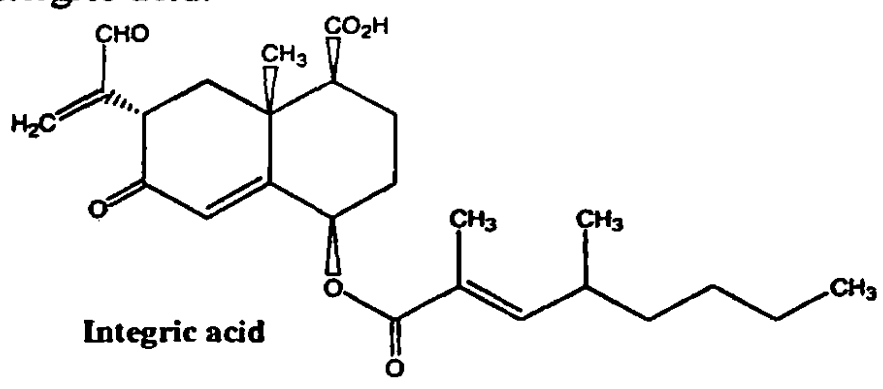
In a program to investigate antimicrobial activities of Sri Lankan fungi we have collected and screened the organic extracts of 18 fungal species (growing either on decaying tree or on soil) from the Gampaha district. The combined methanol/ dichloromethane extracts of these fungi were tested against four bacterial species (*Escherichia coli, Staphylococcus aureus, Bacillus sp.* and *Klebsiella aerogenes*) and four fungal species (*Rhizoctonia solani, Curvularia sp, Corynuspora cussicola, Colletotrichum gloeosporioides). Xylaria hypoxylon* which was collected growing on a decaying jak tree showed selective activity against *K. aerogenes*. Therefore bioassay guided fractionation was undertaken to isolate the active compound(s). The crude extract (700 mg) of *X. hypoxylon* was chromatographed on silica gel (2.5 cm × 12 cm) initially using gradient elution (starting with hexane/ethyl acetate; 64:36 and the polarity gradually increased up to ethyl acetate) to elute sequentially the fractions **A**, **B** and **C**. Next, the column was washed with methanol to elute fraction **D**. Fractions **C** and **D** showed activity against *K. aerogenes*. Fraction **C** was purified further on a Waters Sep-Pak® 12CC (2 g) Silica Cartridge using gradient elution with hexane/ EtOAc mixtures. The ^1H NMR spectrum of purified **C** showed the presence of four methyl groups, four olefinic protons and an aldehyde group among other signals. The high resolution mass spectrum proved the molecular formula of **C** to be $C_{25}H_{34}O_6$. Analysis of 2D NMR data proved the structure of **C** to be intergric acid.



Integric acid

Intergric acid has previously been isolated from an unidentified *Xylaria* species as an inhibitor of HIV-1 integrase enzyme.[1] However, this is the first time the antimicrobial activity of this compound is reported. In the disk diffusion assay against *K. aerogenes*, intergric acid gave an inhibition zone of 3 mm at a concentration of 50 µg per disk. The ^1H NMR spectrum of the polar fraction **D** showed it to be a mixture of compounds with the major component exhibiting two prominent triplets at ä 3.65 and ä 3.78 indicating the presence of a taurine type metabolite. No further work was done on this fraction.

References

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Competition of drugs to serum albumin: study of binding potency of salicylic acid and its dinitro derivative to albumin

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The interaction of bovine serum albumin (BSA) with positively charged salicylic acid (SA) and 3,5 dinitrosalicylic acid (DNS) in aqueous buffer solution at physiological pH was studied with UV-visible and fluorescence spectroscopy to evaluate the effect of substituents in SA towards the binding on BSA. It was found that SA and DNS could interact and induce conformational change of BSA in both ground and excited state.

The fluorescence studies were carried out at three different temperatures, 298, 303 and 308 K to evaluate the binding mode, the binding constant and the protein structure changes of BSA in the presence of SA and DNS in aqueous solution at pH 7.45. The quenching constants K_q, K_{sv} and the association constant K were calculated according to Stern–Volmer equation

based on the quenching of the fluorescence of BSA. The K_{sv} values obtained for SA and DNS was 25.18 × 10^3 and 89.5 × 10^3 dm^3 mol^{-1} respectively. Binding constants (K) obtained for SA and DNS at 308 K were 0.24 × 10^3 and 3.1 × 10^3 respectively. This clearly indicates that DNS has more potency towards the BSA relative to SA at physiological pH.

The thermodynamic parameters, the enthalpy ($\ddot{A}H$) and the entropy change ($\ddot{A}S$) for SA were estimated as -287.13 J mol^{-1} and -748.23 J $mol^{-1}K^{-1}$ at pH 7.45 and 308 K. For DNS, $\ddot{A}H$ and $\ddot{A}S$ were estimated as -136.30 $kJmol^{-1}$ and -0.36 $Jmol^{-1}$ respectively by using the van't Hoff equation. These results show that DNS is a stronger quencher than SA and has a stronger affinity towards BSA. Because of the electron withdrawing effects of the nitro groups, DNS largely exists as its anionic form which enhances the binding capacity to the macromolecule. This phenomenon could explain the greater cytotoxicity of DNS in cells compared to that of SA. From fluorescence and UV-vis spectra of binding of SA and DNS to BSA, it can be concluded that the dielectric environment of the chromophore of the protein has been more exposed towards the hydrophilic region upon change of the microenvironment of the molecule.

Treatment of textile effluents using seawater – A low cost treatment method

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Most of the dyes used in the textile dyeing industry show a high degree of chemical and photochemical stability. One of the reasons for the colour of textile effluents is the presence of colloidal particles of dyes and pigments. Colloids having a surface charge cannot aggregate under normal conditions due to electrostatic repulsions. Repulsion between colloidal particles can be decreased by coagulation. As a result, colloidal particles aggregate and form flocks. Formation of flocks initiates sedimentation, due to their large size and weight. Electrostatic repulsions between colloidal particles can be further decreased by increasing the ionic strength and by adjusting the pH of the medium. Thus coagulation can be enhanced by addition of an electrolyte with the coagulating agent and by controlling the pH. Seawater is a cheap source for Mg^{2+}, Ca^{2+} and Na^{+}ions.[1] Suspended colloidal particles in waste-water are stabilised by negative charges on their surfaces causing them to repel each other. Cationic coagulants provide positive electric charges to reduce the negative charge of these colloids. As a result these colloid particles can aggregate and form flocks. Release of coloured textile effluents to the environment is not acceptable. Adsorption followed by aggregation and sedimentation using coagulating agents is currently used as a treatment method for textile effluents. Recommended levels for various parameters, those which are allowed to be present in textile effluents, are available (*e.g.* pH = 6.5-8.5, TSS = 50 ppm, BOD = 60 ppm and COD = 250 ppm). A textile industry, located 2 km close to the sea was selected for the present study and it was reported that its running cost is high due to use of alum (2 g L^{-1}) as the coagulating agent for treatment of the effluent.

The present study indicates that, when the pH of the textile effluent was adjusted to e"11 by using lime and then treated with 2.5% of seawater (0.0810 mol L^{-1} of total Mg^{2+} and Ca^{2+} ions) the turbidity, TSS, COD and BOD_5 could be reduced by 99, 97, 58 and 62% respectively. Possible reasons for the above reductions are, lime can act as a coagulating agent in addition to making the medium alkaline, and sea water enhances coagulation by increasing the ionic strength. After the above treatment, as pH, COD and BOD_5 were still higher than the recommended levels, it was further treated with alum (0.05%), which reduced the pH and BOD_5 to recommended levels. This is due to the fact that alum is a good coagulating agent and it can reduce the pH of the medium. The COD was still high and it was lowered to the recommended level by aerating the supernatant liquid. The colour was also considerably reduced due to this treatment.

In this method the amount of alum used is 75% less, when compared to the previously used method & it is economically feasible because seawater is abundantly available.

References

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