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DEVELOPMENT OF A METHOD TO DETECT INHIBITON OF GLYCATION INDUCED PROTEIN CROSS-LINKING BY MEDICINAL PLANTS

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Protein glycation plays a key role in the development of chronic complications associated with diabetes mellitus such as atherosclerosis, nephropathy retinopathy and neuropathy. During later events of glycation, advanced glycation end products (AGEs) are formed and some AGEs form protein cross-links. Cross-linking of extracellular matrix proteins, especially collagen and elastin, increases vascular stiffness and diminishes vascular compliance leading to organ damage. Inhibition of AGEs formation is one of the therapeutic approaches to prevent the progression of complications due to diabetese. Studies conducted in Sri Lanka in this regard are extremely limited. The objective of this study was to establish a method to identify plant based inhibitors of glycation induced protein cross-linking and to assess whether *Coriandrum sativum, Kalanchoe laciniata* and *Murraya koenigi* delay or prevent glycation induced protein cross-linking.

Lysozyme was incubated at 37°C up to 4 weeks with different concentrations of glucose, fructose and ribose in sodium phosphate buffer (pH 7.4). Appropriate controls and blanks were carried out. Aminoguanidine (AG) was used as the standard inhibitor. Extracts of *Coriandrum sativum* ('Coriander') seed, *Kalanchoe laciniata* ('Akkapana') leaves and *Murraya koenigi* (curry leaf) leaves shown to have the property of protein glycation inhibition, using a different method conducted by us were used. Aliquots were removed from the incubation mixtures at intervals and analyzed for the presence of AGE induced protein cross-links using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). Appearance and the intensity of high molecular weight products were assessed.

We have demonstrated dimer, trimer and tetramer formation as a result of glycation induced protein cross-linking. Extent of cross-linking was dependent on sugar concentration. Ribose seems to promote cross-linking at much lower concentrations. Among the sugars used, cross-linking was slowest in the presence of glucose and fastest in the presence of ribose. AG inhibited glycation induced protein cross-linking in presence of all three sugars. *Coriandrum sativum* ('Coriander') seed, *Kalanchoe laciniata* ('Akkapana') leaves and *Murraya koenigi* (curry leaf) leaves inhibited protein cross-linking in presence of both glucose and fructose. This inhibition was greater than that of AG.

Analytical techniques available to identify protein glycation inhibitors require expensive specialized equipment. We report a novel and simple method that is suitable to screen medicinal plants for their effect on glycation induced protein-cross linking. However, in this study, we were unable to identify the stage at which this inhibition took place. In conclusion, we have developed a simple SDS-PAGE method to identify plant based inhibitors which can delay or prevent glycation induced protein cross-linking. We also demonstrated the effectiveness of *Coriandrum sativum* seed, *Kalanchoe laciniata* leaves and *Murraya koenigi* leaves in delaying glycation induced protein cross-linking *in vitro*.

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