NOVEL α- GLUCOSIDASE INHIBITORS FROM LICHEN *Cladonia* sp.

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Introduction

Herbal medicine texts have reported several species of lichens including the reindeer lichens such as *Cladonia* sp. (Perez-Llano, 1944). Reindeer lichens have been taken to treat fever, jaundice constipation, convulsions, coughs, and tuberculosis (Brown, 2001). *Cladonia pyxidata* (L.) Hoffm. is an useful remedy for whooping cough (Chevallier, 1996).

The medicinal utility of lichens is attributed to the presence of characteristic and unique secondary lichen metabolites. However, much work remains to link medical effects with specific lichen metabolites. Furthermore, these distinct classes of metabolites have never been tested for their enzyme inhibitory assay against the α-glucosidase.

Glucosidase enzymes are involved in several biological processes, such as the intestinal digestion, the biosynthesis of glycoproteins, and the lysosomal catabolism of the glycoconjugates. Intestinal α-glucosidase is involved in the final step of the carbohydrate digestion to convert these into monosaccharides which are absorbed from the intestine. As a result of catalysis produced by α-glucosidase enzyme in the final step of the digestive process of carbohydrates, its inhibitors can retard the uptake of dietary carbohydrates and suppress postpandial hyperglycemia, and could be used to treat diabetics and or obese patients.

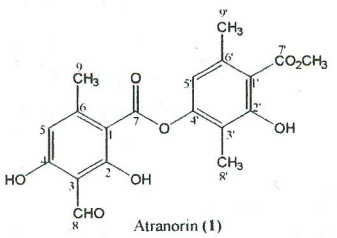
In continuation of our research on Sri Lankan lichens, the reindeer lichen *Cladonia* sp. collected Labukella region was chemically investigated. Isolated compounds were subjected for the α-glucosidase inhibitory assay.

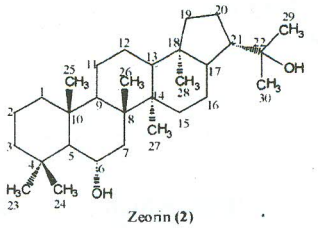
Materials and Methods

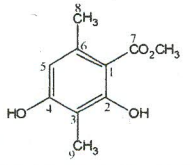
Isolation and Identification of Lichen metabolites

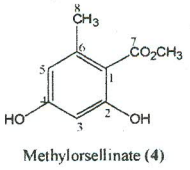
Cladonia sp. described in this study was collected from the rocks of Labukella, Central Province, Sri Lanka. Manually cleaned, air-dried, crushed lichens were sequentially extracted with CH_2Cl_2, followed by MeOH. The crude CH_2Cl_2 and MeOH extracts were fractionated *via* Medium Pressure Liquid Chromatography (MPLC), and column chromatography using silica gel 60. Identification of the compounds was carried out using ^1D, ^2D, and mass spectral data. Spectroscopic (^1H and ^{13}C) data were consistent with those of literature reports or authentic samples.

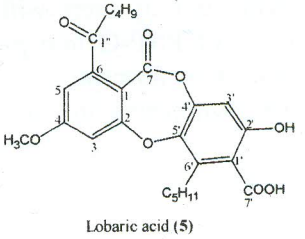
Chromatography of the CH_2Cl_2 extract yielded atranorin (**1**) (145 mg, 0.29 %), zeorin (**2**) (0.52 %), methyl-β-orcinolcarboxylate (**3**) (0.08 %) whereas the MeOH extract afforded methyl orsellinate (**4**), (0.02 %) and the depsidone lobaric acid (**5**)(0.37 %).











α-Glucosidase inhibitory assay

The α-glucosidase inhibition assay was performed according to the method of Matsui *et al.*, (2001). The inhibition of test compounds **1, to 5** (5 µL, 1 mM) was measured spectrophotometrically at pH 6.9 and at 37 °C using *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as the substrate and the enzyme, in sodium phosphate buffer containing 100 mM NaCl. Acarbose and 1-Deoxynojirimycin were used as positive controls.

All the reactions were performed in triplicates in 96-well microplates. The results (change in absorbance per min.) were processed using ELISA (multiple reader Spectra Max Plus - Molecular Devices). The IC_{50} values of the compounds were calculated using EZ-Fit Enzyme kinetics software program.

Results and Discussion

Cladonia sp. described in this study belongs to the family Cladoniaceae which is a common and large family with over 70 species known. α-Glucosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer and lysosomal storage disease(Blidi *et al.*, 2006). However prior to this study there were no reports on α-glucosidase inhibitory activities of lichen compounds.The α-Glucosidase inhibition assay was performed using *p*-nitrophenyl α-D- glucopyranoside (PNP-G) as a substrate. The increments in absorption at 400 nm due to the hydrolysis of PNP-G into *p*- nitrophenol by α- glucosidase was

monitored continuously with a molecular devices spectrophotometer.

Table 1. % Inhibition or IC_{50} values of some lichen compounds against α-glucosidase

Sample	% Inhibition	IC_{50} ± SEM [μM]
Zeorin (**2**)		100.0 ± 0.3
Methyl-β-orcinol carboxylate (**3**)		140.0 ± 0.6
Methyl orsellinate (**4**)		165.0 ± 1.2
1-Deoxynojirimycin		**425.0 ± 8.9**
Acarbose		**700.0 ± 10.4**

In the case of test compounds which inhibit the enzyme activity there will be less hydrolysis of PNP-G into *p*-nitro phenol and less absorption at 400 nm. 1-Deoxynojirimycin and acarbose were used as positive controls (Matsui *et al.*, 2001).

The lichen specific triterpenoid zeorin (**2**) showed an IC_{50} value of 100.0 ± 0.3, which is seven times less than that of the standard acarbose and four fold lower than that of the standard 1-deoxynojirimycin. The two simple aromatic compounds namely methyl orsellinate (**4**), and methyl-β-orcinol carboxylate (**3**) also showed IC_{50} values several fold less than even those of the standards 1-deoxynojirimycin and acarbose (Table 1). Excellent activities shown by the above mentioned structurally different lichen metabolites, makes further studies of lichen metabolites against α-glucosidase worth while. However, atranorin (**1**), and lobaric acid (**5**) were inactive.

References

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