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**SHORT COMMUNICATION**

# Potent α-glucosidase inhibitors from the lichen *Cladonia* species from Sri Lanka

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**Abstract:** The discovery of *α*-glucosidase inhibitors has been actively pursued with the aim to develop therapeutics for the treatment of diabetes and other carbohydrate-mediated diseases. This study focused on the lichen *Cladonia* sp., which yielded three potent *α*-glucosidase inhibitors, namely zeorin (1), methyl *β*-orcinolcarboxylate (2) and methylorsellinate (3) with several fold higher inhibitory activities than those of acarbose, an anti-diabetic drug used to manage type II diabetes mellitus and the standard, 1-deoxynojirimycin. Atranorin (4) and lobaric acid (5), the other two metabolites isolated from the lichen did not show any α-glucosidase inhibitory potential. All compounds were identified on the basis of one dimentional (1D) and two dimentional (2D) NMR spectral data and with comparison to reported data.

**Keywords**: *α*-Glucosidase inhibitory activity, diabetes type II, lichen metabolites, methyl *β*-orcinolcarboxylate, methylorsellinate, zeorin.

## INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disease affecting about 173 million people (2.8 %) worldwide and is expected to reach 4.4 % (366 million) by 2030 (Elya *et al*., 2012). The prevalence estimates of DM are high for all Asian countries and are expected to double in the next two decades. Sri Lanka is also expected to face a high burden of DM in the coming years (Katulanda *et al*., 2008).

This situation has prompted numerous efforts to investigate for new therapeutic agents to stem the progress of diabetes. One of the strategies in the management of DM, in particular non-insulin-dependent DM, is to inhibit the absorption of carbohydrates. Intestinal *α*-glucosidases are involved in the final step of the carbohydrate digestion, converting them into monosaccharides. Hence, the inhibitors of *α*-glucosidases retard the liberation of D-glucose, resulting in the suppression of postprandial hyperglycemia and therefore, are used as an effective treatment of type II diabetes and obesity (Raskin *et al*., 2007).

*α*-Glucosidases are also effective in lowering the insulin release, which leads to the lowering of plasma lipids. The current interest in *α*-glucosidase inhibitors has been extended to a diverse range of diseases including lysosomal storage disorders, cancer and AIDS. In addition, *α*-glucosidase inhibitors have also been used as antiobesity drugs, fungistatic compounds, insect antifeedants, antivirals and immune modulators (Thadhani *et al.*, 2011 b)

Natural products possess great structural diversity and are an attractive source to investigate for finding novel chemotypes to control hyperglycaemia. One of the most potent hyperglycaemic active plants native to Sri Lanka and India is *Salacia reticulata* Wight (Kotalahimbutu: Sinhalease). Although its rich chemistry of organic-soluble extracts was investigated in Sri Lanka (Gunatilaka *et al*., 1993; Tezuka *et al.*, 1993; Tezuka *et al.*, 1994; Dhanabalasingham *et al.*, 1996) the potent *α*-glucosidase activity of the aqueous extract was reported by a group of Japanese researchers (Yoshikawa *et al.*,

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1997). In addition, various plant metabolites such as flavanoids, alkaloids, terpenoids, saponins, anthocyanins, curcumnoids, phenolic compounds etc., have shown potent $\alpha$-glucosidase inhibitory activity (Kumar *et al.,* 2011). The saponin rich Sri Lankan plant *Cyclea burmanni* Arn. ex Wight remains unexplored for its antidiabetic activity (Bandara *et al*., 1989). More than ca. 1200 plant species have been recorded to be used empirically worldwide for their alleged hypoglycaemic activity (Tundis *et al.,* 2010). Higher plants still continue to be a rich source for compounds with potential antidiabetic activity. Besides, lichens have been largely unexplored for metabolites possessing antidiabetic activity. Interestingly, tropical lichens, particularly those from Sri Lanka, continue to yield a wide variety of biologically active compounds, and also serves as a rich source of new lichen species (Orange *et al.*, 2001; Karunaratne *et al.*, 2005; Choudhary *et al.*, 2009; Williams *et al.*, 2011; Thadhani *et al.*, 2011a & 2012; Jayalal *et al.*, 2012). In the present study, the focus was on the constituents from the lichen *Cladonia* sp. of Sri Lankan origin with potent $\alpha$-glucosidase inhibitory activity (Thadhani *et al.*, 2011b). *Cladonia* sp. used in this study belongs to the family Cladoniaceae, which is a large family with over 70 known species (Ahti *et al*., 1993). Northern Native American people used *Cladonia* sp. in medicinal teas to treat colds, fevers, jaundice, and as poultice to relieve the ache of arthritic joints, for the treatment of convulsions, coughs, and tuberculosis (Brown, 2001).

## METHODS AND MATERIALS

*Cladonia* sp. specimens were collected from the rocks of Labukella, Central Province, Sri Lanka. A voucher specimen was identified and deposited at the National Herbarium, Peradeniya (pdnA 3 4 10 4). The lichen specimens were cleaned, washed and air-dried at room temperature, and sequentially extracted into $CH_2Cl_2$ and MeOH. Silica gel column chromatography of the $CH_2Cl_2$ extract of the lichen yielded zeorin (1) (0.52 %), methyl $\beta$-orcinolcarboxylate (2) (0.08 %), atranorin (4) (0.29 %), and the MeOH extract afforded methylorsellinate (3), (0.02 %) and the depsidone lobaric acid (5) (0.04 %) on the dry weight basis of the lichen. All compounds were identified on the basis of 1D and 2D NMR spectral data and comparison with the reported spectral data (Huneck & Yoshimura, 1996).

The $\alpha$-glucosidase inhibition of the isolated compounds (5 µL, 1 mM) was measured spectrophotometrically at pH 6.9 and at 37 °C using *p*-nitrophenyl-$\alpha$-D-glycolpyranoside (PNP-G) (0.5 µM) as the substrate and 250 units/ mL of enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl, acarbose (0.78 mM) and 1-deoxynojirimycin (0.425 mM) were used as positive controls (Oki *et al.*, 2000). The increments in absorption due to the hydrolysis of PNP-G by $\alpha$-glucosidase were monitored continuously with a spectrophotometer at λ 400 nm. All reactions were performed in triplicate in

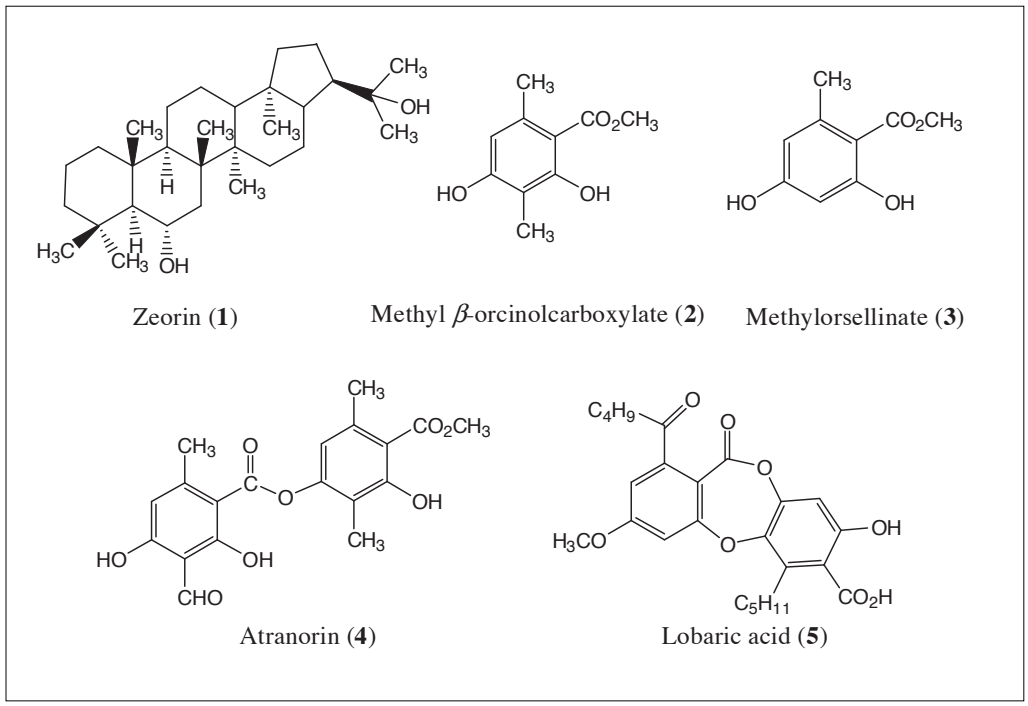


**Figure 1:** Structures of compounds (1) – (5) isolated from *Cladonia* sp.

96-well microplates. The results were processed using ELISA (multiple reader Spectra Max Plus -384, and 3400 Molecular Devices, CA, USA). The $IC_{50}$ values of the compounds were calculated using EZ-Fit Enzyme kinetics software programme (Perrella Scientific Inc., Amherst, USA).

## RESULTS AND DISCUSSION

From the isolated compounds 1 - 5, triterpenoid zeorin (1) showed the highest *α*-glucosidase inhibitory activity with an $IC_{50}$ value of 100.0 ± 0.3 µM, which is seven times lower than that of acarbose, ($IC_{50}$ = 700.0 ± 10.4 µM), a widely prescribed drug in the management of type II diabetes, and four fold more active compared to the potent *α*-glucosidase inhibitor, 1 - deoxynojirimycin ($IC_{50}$ = 425.0 ± 8.9 µM). The two phenolic compounds, methyl *β* - orcinolcarboxylate (2) ($IC_{50}$ = 140.0 ± 0.6 µM) and methylorsellinate (3) ($IC_{50}$ = 165.0 ± 1.2 µM) also exhibited 4 - 5 fold higher activity than acarbose. Importantly, neither zeorin (1) nor methylorsellinate (3) showed any cytotoxicity in the brine shrimp lethality assay (Thadhani *et al.,* 2012), thus highlighting its potential use as a drug candidate. However, methyl *β* - orcinolcarboxylate (2) showed moderate cytotoxicity with a $LD_{50}$ value of 17.07 µg/mL as compared to the standard etoposide ($LD_{50}$ = 7.43 µg/mL). It is noteworthy that kotanalol and salacinol, the two well-known *α*-glucosidase inhibitors isolated from *S. reticulata*, showed only a maximum of 1 - 1.5 fold *α* -glucosidase inhibitory activity compared to the standard drug acarbose (Yoshikawa *et al.,* 1997). However, the other two compounds isolated from the lichen, namely atranorin (4) and lobaric acid (5) showed no *α*-glucosidase inhibitory properties.

Methylorsellinate or 2,4-dihydroxy-6-methylbenzoate (3) has been reported from various lichens such as *Parmotrema grayana*, *Roccella montagnei* and *Pseudocyphellaria crocata*. Methyl *β*-orcinolcarboxylate or 2,4-dihydroxy-3,6-dimethyl benzoate (2) is a common lichen metabolite reported from *Stereocaulon alpinum* Laur., etc. Zeorin or 6, 22-hopanediol (1) is a common constituent of various lichens, i.e. *Heterodermia*, *Anaptychia*, *Lecanora*, *Parmelia*, *Nephroma* and *Placodium* sp. Although zeorin (1) is one of the most common lichen specific metabolites, no bioactivities of this compound have been reported before to the best of our knowledge. Various bioactivities of methyl *β*-orcinolcarboxylate (2) have been reported including anti-cancer activities (Huneck, 1999), while methylorsellinate (3) exhibits antibacterial and antifungal activities (Thadhani *et al.,* 2012). Significantly, this is the first report on the *α*-glucosidase inhibitory activity of lichen compounds.

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