Isolation and structure elucidation of serrulatane diterpenoids from the roots of Eremophila longifolia

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Isolation and structure elucidation of serrulatane diterpenoids from the roots of *Eremophilia longifolia*

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**Abstract**

The incidences of type 2 diabetes (T2D) are estimated to increase by 51% by 2045. Aerial parts of the genus *Eremophila* have shown to be a rich source of serrulatane diterpenoids which have shown antihyperglycemic activity. In this study, roots of *Eremophila longifolia* were extracted with dichloromethane and methanol (1:1) and the extract screened for bioactivity against α-glucosidase, α-amylase, and PTP1B. Subsequently, the extract was investigated using LC-PDA-HRMS for dereplication and a combination of analytical-scale HPLC and NMR spectroscopy for structure elucidation. Eight serrulatane diterpenoids were identified, of which six were previously unreported. This work demonstrates *Eremophila longifolia* root material to be a rich source of structurally diverse serrulatane diterpenoids.

**Background**

The global prevalence of diabetes mellitus is estimated to rise from 463 million incidences in 2019 to 700 million incidences in 2045, i.e., an increase of 51%. Type 2 diabetes (T2D) accounts for 90% of all diabetes worldwide [1]. T2D is characterized by a combination of resistance to insulin action and reduced pancreatic insulin production. Current treatments constitute a combination of lifestyle changes and the use of antihyperglycemic drugs to control blood glucose levels [2]. α-Glucosidase and α-amylase belong to the carbohydrate-degrading enzymes which break down polysaccharides into monosaccharides after a meal [3]. Another target-enzyme, protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the insulin signalling pathway by dephosphorylating the insulin receptor [4]. *Eremophila* is an endemic genus to Australia and consist of approximately 230 species [5]. Some *Eremophila* species contain longifolia (R.B. F.Muell. (Figure 2) were used by the Australian Aboriginal peoples for medicinal and cultural purposes [6]. Aerial parts of *Eremophila* have shown to be a rich source of serrulatane diterpenoids with antioxidant, anticancer, and antihyperglycemic activity [7-9].

**Method**

The root bark was extracted with dichloromethane and methanol (1:1) and screened for α-glucosidase, α-amylase, and PTP1B inhibitory activity after separating the extract into microfractions from analytical-scale high performance liquid chromatography (HPLC). The results (percentage inhibition) from bioassaying of each fraction were plotted at their respective time result, resulting in a triple high-resolution inhibition profile. The extract was investigated using liquid chromatography – photo diode array high-resolution mass spectroscopy (LC-PDA-HRMS) for dereplication and a combination of HPLC and NMR spectroscopy for structure elucidation.

**Findings**

The constructed biocompatible graph (Figure 3) displays inhibitory activity against the targeted enzymes for some of the peaks, and HPLC-PDA-HRMS-based dereplication indicated the presence of previously unreported compounds. Repeated analytical-scale HPLC separation of the material eluted with peak 2, 4, 9-13, 16, and 18 led to isolation of six previously unreported serrulatane diterpenoids (Figure 1) and two previously reported. UV, HRMS and ‘H NMR led to identification of 9 as Elisabatin A and 11 as Elisabatin B, respectively. Compounds 2, 4, 9, 12, 16, and 18 are all serrulatane diterpenoids. In addition to having the same skeleton, all compounds have an oxygen-atom in degrading position 3, 7, and 8. Compound 2 is the only compound with a ketone on the sidechain, and 4 is the only compound to have a ketone at position 3. 10 has an exocyclic ring system, which too our knowledge have not been isolated from *Eremophila* before. Compound 12 shows structural similarity with 2, but has an additional oxygen-atom instead of a ketone at position 14. 16 is the only compound with a tetrahydropyran and 18 has a tetrahydropyranylen together with a cyclopentane.

**Conclusion**

This work demonstrates *Eremophila longifolia* root material to be a rich source of structurally diverse serrulatane diterpenoids, some of which appear to have inhibitory activity against diabetes target-enzymes. This needs to be tested with concentration-dependent inhibition of the respective enzymes.

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