

ASSESSING THE BIOACTIVITIES OF POLYPHENOLS FROM SEAWEEDS

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INTRODUCTION

Edible seaweeds contain a range of potentially bioactive components including polyphenols called phlorotannins. Extracts from edible seaweeds such as Dulse (*Palmaria palmata*) have been shown to be effective antioxidants and capable of inhibiting cancer cell proliferation (1). Seaweed phlorotannins are structurally similar to soft fruit tannins which are effective inhibitors of alpha-amylase, a crucial enzyme in starch degradation which has been proposed to influence post-meal blood glucose levels in patients with non-insulin dependant Diabetes mellitus (NIDDM) (2). The main aim of this project was to extract polyphenols from four edible seaweeds (*Laminaria digitalis*, *Ulva lactuca*, *Palmaria palmatum*, and *Ascophyllum nodosum*, clockwise from top left) to assess their potential as antioxidants, for future studies to assess bioactivities such as anti-proliferative effects on cancer cells, and to assess activities against digestive enzymes.

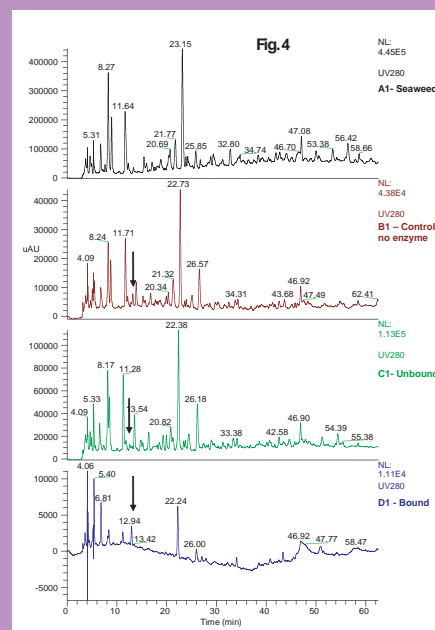
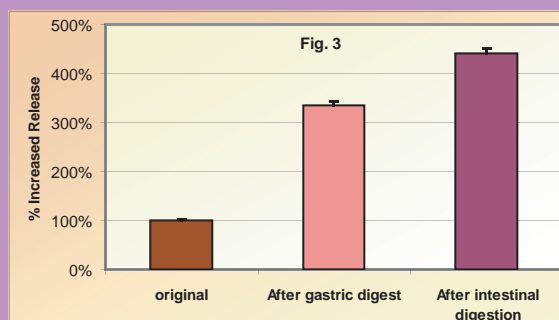
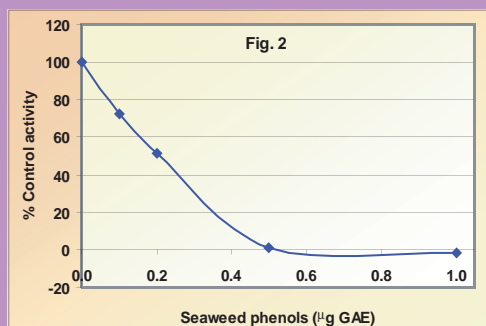
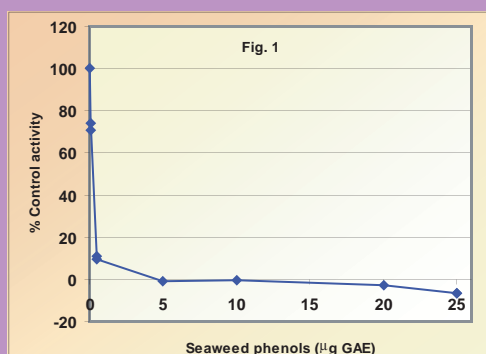


RESULTS

Inhibition of alpha-amylase

Initial attempts to extract polyphenols from the four seaweed samples were unsuccessful. Only the *Ascophyllum nodosum* extract produced sufficient material for amylase inhibition assays. At 25 mg/ml, this extract caused complete inhibition of amylase activity (Fig. 1) and near complete inhibition at 0.5 µg/ml (Fig. 2). Approximately 0.2 µg/ml of seaweed extract caused 50% inhibition, giving a K_i value 100-fold lower than previous work with green tea (2). Increased amounts of polyphenols were released by an in vitro simulation of gastro-intestinal digestion (Fig. 3) which confirms that these compounds would be released when the seaweed as eaten.

The amylase was inhibited by the seaweed extract even when immobilized via an anti-amylase antibody to an ELISA plate and this provided a means to attempt to trap the active amylase inhibitors. Polyphenolic components that bound (Fig. 4, D1) or did not bind (Fig. 4, C1) to the immobilized amylase were compared to the original seaweed extract and a no-amylase binding control (Fig. 4, A1 & B1) using liquid chromatography-mass spectrometry (LC-MS). Multiple candidate compounds were indicated with the peak labelled with the arrow as a good possibility. However, none of these compounds could be identified and it is also possible that more than one compound is responsible for the inhibition.



DISCUSSION

The extract from *Ascophyllum nodosum* inhibited α-amylase at concentrations 500-times lower than extracts from soft fruit such as raspberry (2). At these very low levels, the active components may have biological effects relevant to blood glucose control and type 2 diabetes or even the prevention of dental caries. That these compounds were released during simulated human digestion was important for these putative biological effects. Although candidate

compounds were indicated, the nature of the inhibitory components are not known. Future work will include activity driven fractionation of the extract using large-scale HPLC or the use of an immobilized anti-amylase antibody column to trap the inhibitor-enzyme complex. Although it may seem strange that seaweeds contain a potent inhibitor of a human digestive enzyme, seaweeds have evolved a range of anti-feedant compounds to protect against marine herbivores such as molluscs (3). The seaweed polyphenol samples obtained will also be screened for a range of other bio-activities ranging from anti-proliferative effects on cancer cells to inhibition of other digestive enzymes

BACKGROUND

1. Yuan et al. (2005) Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation in vitro. Food and Chemical Toxicol. 43: 1073-81
2. McDougall et al. (2005) Different polyphenolic components of soft fruits inhibit amylase and glucosidase. J. Agric. Food Chem. 53: 2760-66
3. Pavia & Toth. (2000). Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. Ecology 81: 212-25