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# Application of an online post-column derivatization HPLC-DPPH assay to detect compounds responsible for antioxidant activity in Sonchus oleraceus L. leaf extracts

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# 1. Introduction

# Introduction

The consumption of antioxidants in foods or nutritional supplements has been associated with prevention of oxidative damage caused by excessive free radicals. Synthetic antioxidants, such as butylated hydroxytoluene, have widely been used to prevent deterioration of foodstuffs by inhibiting oxidation. However, studies have implicated synthetic antioxidants in carcinogenesis and hepatotoxicity.

# Introduction

Studies of in-vitro antioxidant activity have been commonly conducted on extracts prepared from plants and assessed by the DPPH assay. DPPH is a stable free radical with a deep purple colour that changes to pale yellow upon reduction. Reducing activity is defined as the concentration of antioxidant required to scavenge the initial DPPH concentration by 50%.

# Aim

To use an online assay to identify key antioxidants in Sonchus oleraceus leaf extracts and to investigate the effect of leaf position and extraction conditions on antioxidant concentration and activity.

# 2. Materials and Methods

# 2.1 Plant material

- S. oleraceus seeds were collected from plants growing wild in Oamaru, New Zealand.Seeds were germinated in a 50 : 50 potting compost.Tap water was supplied every 2 days.

# 2.2 Preparation of extracts for isolation and identification

Fresh S. oleraceus leaves (170 g) were freeze–dried and ground into powder. Analytical HPLC was used to confirm peaks in the eluted fractions. Fractions with compounds of interest were freeze–dried and analysed by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

# 2.3 Preparative HPLC method

A sample of the dried extract was dissolved in 3% formic acid before isolation of the main compounds with anti-oxidant activity for identification by preparative HPLC.

# 2.4 Statistical analysis

Data obtained from different leaf positions and different extraction conditions are presented as mean values SD, expressed as μmol compound per 100 mg dry weight.

# 3. Results

# 3.1 Identification of antioxidant compounds

Three main peaks with corresponding free-radical scavenging activity were identified from S. oleraceus leaf extracts using the online HPLC-DPPH radical assay (Figure 1), with peak 3 being present in the highest proportion.

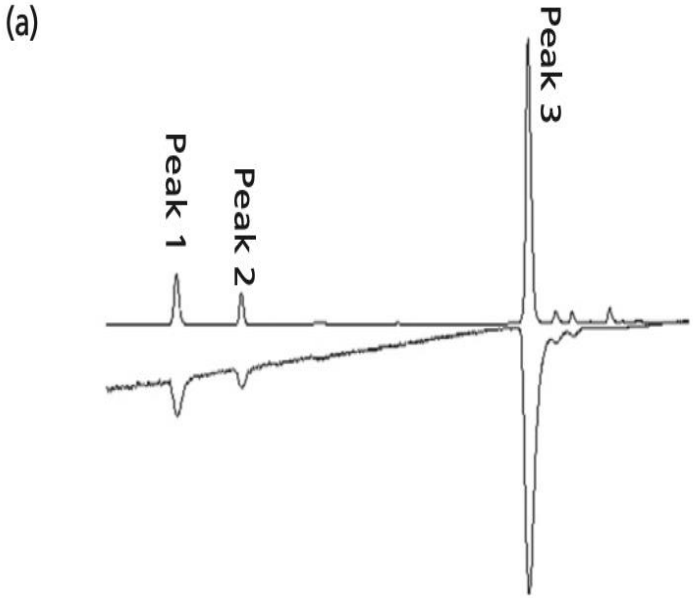


Figure 1 Typical chromatogram of Sonchus oleraceus leaf extract obtained from the post-column HPLC-DPPH radical method.

# 3.2 Effect of leaf position on antioxidants

The concentrations of the key antioxidant compounds differed depending on leaf position on the plant (Table 1). Old leaves from the bottom of the plant contained the lowest concentration of the three key antioxidant compounds.

| Positions | Caftaric acid | Chlorogenic acid | Chicoric acid | Total |
|---|---|---|---|---|
| Top | 0.95 ± 0.36 | 0.80 ± 0.37*** | 2.61 ± 0.66** | 4.37 ± 0.89 |
| Middle | 0.93 ± 0.29 | 0.29 ± 0.22 | 3.90 ± 1.55 | 5.12 ± 1.95 |
| Bottom | 0.58 ± 0.33** | 0.25 ± 0.19 | 2.63 ± 1.61** | 3.46 ± 1.97** |

Table (1) The concentration of the three key antioxidants in Sonchus oleraceus leaf extracts from three different positions

# 3.3 Effects of processing conditions on antioxidants

Pre-treatment with liquid N2 did not improve the amount or activity of antioxidants compared with the leaves that were not treated with liquid N2.

Antioxidant yield and activity did not differ between extracts that were freeze dried and those that were processed using rotary evaporation. Similarly, extraction temperature (Figure 2) and time did not change the amount or activity of the three key compounds.

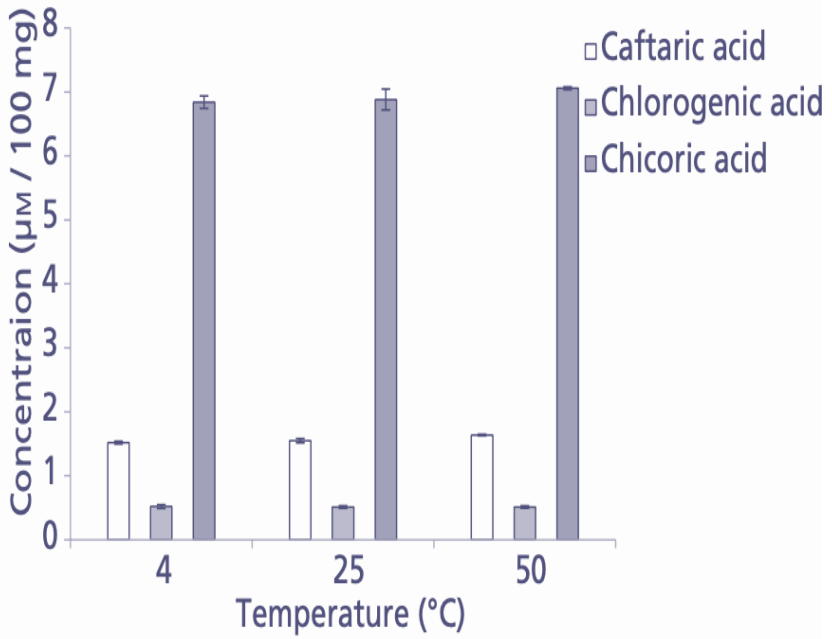


**Figure (2)** Amount of chicoric acid, chlorogenic acid and caftaric acid quantified from Sonchus oleraceus leaf extracts after 2hr extraction time at different temperatures.

# 4. Discussion

# Discussion

Investigation of the antioxidant activity of individual compounds in leaf extracts has previously been achieved by first isolating the compounds of interest followed by a biochemical assay to measure their activity. From the online HPLC-DPPH radical assay, three main peaks with antioxidant activity from S.oleraceus leaf extracts were identified. The three peaks were chicoric acid, chlorogenic acid and caftaric acid as confirmed by NMR and MS analysis, and by comparison with commercial standards as well as literature data. In previous studies, the most commonly isolated antioxidants from S. oleraceus have been flavonoids including luteolin, apigenin, quercetin, kaempferol and their glucosides.

# Discussion

A study on cucumber leaves from different positions showed a lower activity of antioxidant compounds (such as superoxide, ascorbic acid, b-carotene and flavonoids) in the basal leaves compared with leaves from other positions. In Lantana camara L., the highest antioxidant activity was in leaf extracts from the leaves in the middle of the plant. Our results concur with these studies, with the S. oleraceus leaves at the bottom of the plant having the lowest concentration of the three compounds and correspondingly low antioxidant activity.

# 5. Conclusion

# Conclusion

The online post-column derivatization HPLC-DPPH radical assay was found to be useful for screening leaf extracts for individual constituents possessing antioxidant activity. The three main antioxidants in S. oleraceus leaf extracts were shown by NMR and MS analysis to be caftaric acid, chlorogenic acid and chicoric acid.

# References

1- Ou ZQ, Schmierer DM, Rades T, Larsen L, McDowell A.(2012). Application of an online post-column derivatization HPLC-DPPH assay to detect compounds responsible for antioxidant activity in Sonchus oleraceus L. leaf extracts. Journal of Pharmacy and Pharmacology. 65(2):271-279.