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Analytical Methods

# Glucosinolates redox activities: Can they act as antioxidants?

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ABSTRACT

Glucosinolates are a class of secondary plant metabolites particularly occurring in Cruciferae with potential health-promoting properties, as their hydrolysis products, isothiocyanates, possess chemopreventive and antioxidant activities. In the present study, we systematically studied the *in vitro* redox behaviour of 15 glucosinolates, by using a range of analytical methods measuring different activities: (i) radical scavenging activity toward peroxyl and toward ABTS radical (chain-breaking activity); (ii) capacity in modulating the *in vitro* resistance of human low-density lipoprotein (LDL) catalysed by copper (chelating and chain-breaking activity). Data obtained from different assays were compared and analysed by principal component analysis (PCA). PCA allowed us to identify a big cluster of glucosinolates (10 out 15 tested) that do not possess any antioxidant capacity; while, the other five glucosinolates showed moderate and specific antioxidant capacity. Notably, sinalbin and gluconasturtiin were highly active in scavenging ABTS radical and in protecting LDL from copper-catalysed oxidation, respectively. The overall results of this study indicate that just few glucosinolates can act as antioxidants.

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## 1. Introduction

Glucosinolates (GLs) are an important class of secondary plant metabolites particularly occurring in Cruciferae (broccoli, cabbage, kale and Bruxelles sprouts), vegetables commonly grown and consumed worldwide.

GLs are a heterogeneous family of molecules characterised by a similar basic structure containing a sulphur-linked β-D-glucopyranose moiety, a sulphonated oxime group and a side chain derived from different amino acids, which allow their classification in: aliphatic, thio-aliphatic, aromatic and indolic GLs (Fig. 1) (Fahey, Zalcmann, & Talalay, 2001).

GLs have gained growing attention for their potential health-promoting properties, as their hydrolysis products (isothiocyanates, ITCs) are able to induce phase 2 detoxication enzymes and protect animals against chemically induced cancer (Zhang, Cho, Posner, & Talalay, 1992). A large body of available data indicates that the redox activities of ITCs could explain their bioactivity (Zhang et al., 1992). In fact, ITCs exert a indirect antioxidant capacity by modulating phase 2 antioxidant enzymes (Fahey & Talalay, 1999; Valgimigli & Iori, 2009), but they are also able to increase ROS production in cells acting as indirect pro-oxidants (e.g. reacting with protein sulfhydryl groups) (Jacob, Jamier, & Ba, 2011; Valgimigli & Iori, 2009).

Although redox activities of ITCs have been extensively studied, the same has not been systematically done for their precursors GLs. Several studies examine the direct antioxidant properties (radical scavenging and chelating activity) of cruciferous extracts (Biovin et al., 2009; Kurilich, Jeffery, Juvik, Wallig, & Klein, 2002; Sandoval et al., 2002), but just few of them evaluate if and how GLs could contribute to the cruciferous antioxidant capacity, namely if GLs themselves could possess some antioxidant capacity (Cabello-Hurtado, Gicquel, & Esnault, 2012; Pellegrini et al., 2010; Plumb et al., 1996).

Moreover, it has been demonstrated in rats that after oral administration a little fraction of GLs can be absorbed as such (Bheemreddy & Jeffery, 2007); this means that GLs could directly exert their antioxidant activity (if any) into the circulation.

In the present study, we systematically studied the *in vitro* redox behaviour of 15 GLs, belonging to three different chemical classes: aliphatic (gluconapin, sinigrin, progoitrin, epiprogoitrin), aliphatic with an additional sulphur atom (glucoiberin, glucocheirolin, glucoerucin, glucoraphanin, glucoraphenin), aromatic/indolic (glucobarbarin, glucosibarin, glucotropaeolin, sinalbin, glucobrassicin) (Fig. 1). Because an antioxidant may act with different mechanisms, such as scavenging radicals, decomposing peroxides and chelating metal ions, we decided to study the redox behaviour of GLs by using different analytical approaches. In particular we measured: (i) their radical scavenging activity toward peroxyl radical by a competition kinetic procedure and toward radical cation 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) by using TEAC assay and (chain-breaking activity); (ii) their capacity in modulating the *in vitro* resistance of human low-density

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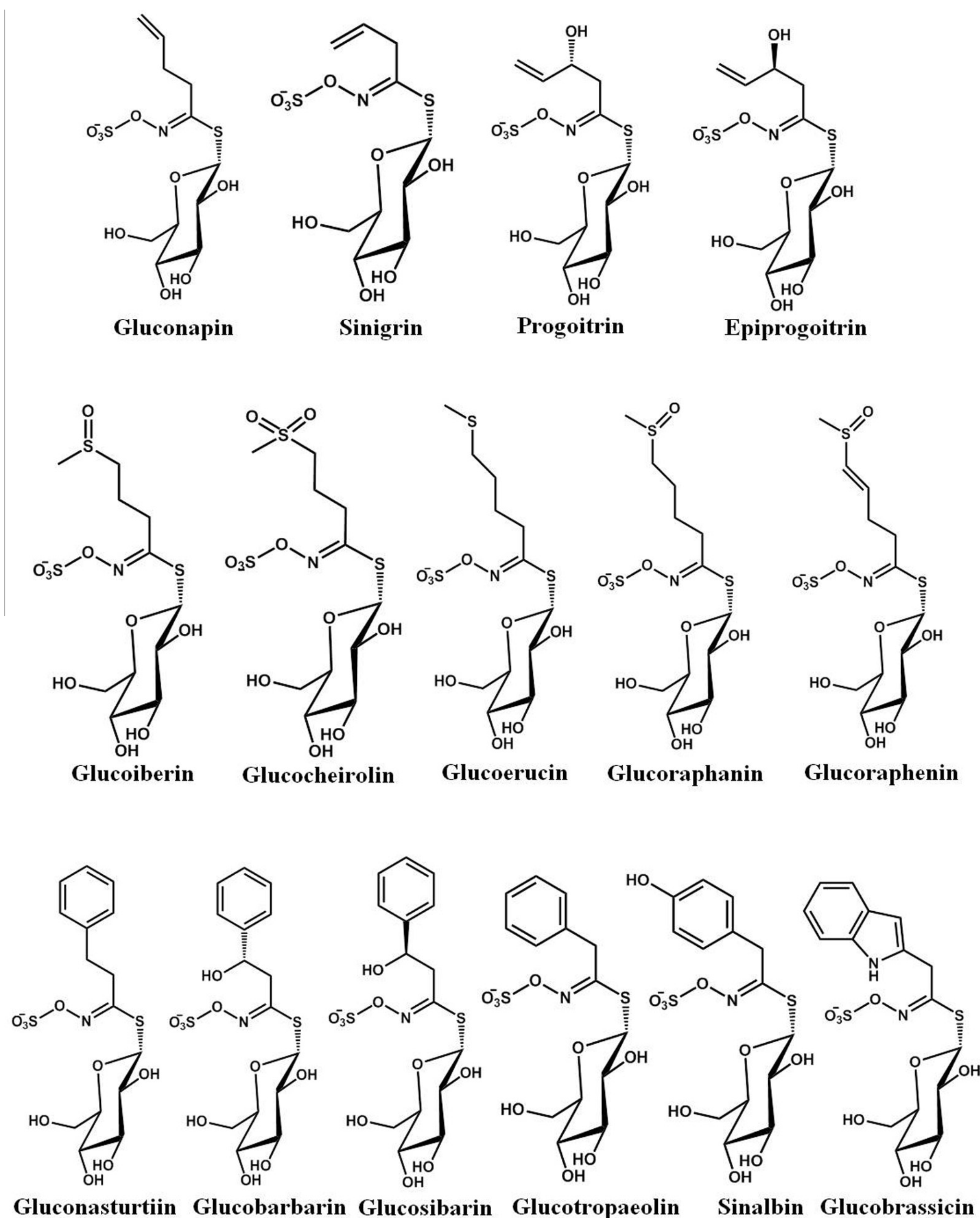


**Fig. 1.** Chemical structure of the studied glucosinolates.

lipoprotein (LDL) catalysed by Cu(II) (chelating and chain-breaking activity).

## 2. Materials and methods

### 2.1. Chemicals

All the GLs were purchased from Phytolab (Vestenbergsgreuth, Germany).

Trolox, ABTS, potassium persulphate, potassium chloride, sodium chloride, hydrogen peroxide and formic acid were from Sigma–Aldrich Chem. Co. (Milwaukee, Wis., USA). Copper chloride was from Fluka AG, Chemische Fabrik (Buchs, Switzerland). 2,2′-Azobis (2-amidinopropane)-hydrochloride (AAPH) was purchased from Polysciences (Warrington, PA, USA). HPLC grade methanol was from Carlo Erba (Milano, Italy).

### *2.2. Peroxyl radicals scavenging activity and copper chelation by a competition kinetic test*

Crocin was isolated from saffron (Bors, Saran, & Michel, 1982). The concentration of crocin was calculated from the absorption coefficient in methanol ($E = 1.33 \times 105\ M^{-1}\ cm^{-1}$ at 443 nm) (Weber, Laskawy, & Grosch, 1974). The competition kinetic test (Tubaro, Ghiselli, Rapuzzi, Maiorino, & Ursini, 1998) derives from that described by Bors et al. (1982). In brief, reaction mixture contained 12 μM crocin and increasing amounts of single glucosinolates (from 25 to 1000 μM) in 10 mM PBS, pH 7.4. The reaction was started by the addition of 10 mM AAPH to the reaction mixture pre equilibrated at 40 °C. The bleaching rate of crocin (Vo), i.e., the rate of its reaction with peroxyl radicals, was calculated by measuring the decrease of its absorption at 443 nm in the first 10 min of reaction. In the presence of increasing concentrations of single GL, the corresponding bleaching rates were termed V.

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