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LC–ESI-MS/MS profile of phenolic and glucosinolate compounds in samh flour (*Mesembryanthemum forsskalei* Hochst. ex Boiss) and the inhibition of oxidative stress by these compounds in human plasma

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ABSTRACT

Samh flour (*Mesembryanthemum forsskalei*) is a foodstuff with high protein content, which can be used as a replacement for wheat flour. It is often consumed by Bedouin tribes of northern Saudi Arabia. Very little is known about bioactive molecules present in samh flour, therefore we analyzed its extracts to evaluate the contents of secondary metabolites. A total of 43 secondary metabolites present in 60% MeOH extract of samh flour were tentatively identified using LC–ESI-MS/MS. These compounds represented five major categories: glucosinolates, sinapic acid and sinapoylglycosides, acylated flavonoids, flavonoids, and amide derivatives. Their effect on oxidative damage of proteins and lipids was determined *in vitro* by assessing levels of protein thiol groups and concentrations of thiobarbituric acid reactive species (TBARS) in human plasma. Obtained results indicated that samh flour is a rich source of compounds with antioxidant activity.

1. Introduction

Mesembryanthemum species (*Aizoaceae* family) are halophytes widely found in semi-arid zones in the northern part of Egypt, Saudi Arabia and Kuwait (Batanouny, 2001). They are used as food and in traditional medicine for treatment of liver diseases, diabetes and ocular infections (Thring & Weitz, 2006; Van Wyk, 2008; Bouftira, Abdelly, & Sfar, 2009; Al-Faris, Al-Sawadi, & Alokail, 2010; Falleh et al., 2011).

Seeds of *Mesembryanthemum forsskalei* Hochst. ex Boiss, also known as forskal fig-marigold, are edible and used for traditional baking in Al-Jouf area of northern Saudi Arabia (Doughty, 1936; Palgrave, 1865). Chemical analysis of samh seeds showed they contain approximately 22% of crude protein, (in comparison to 14% of protein in wheat, 12% in triticale and 8% in rye), about 6% of moisture and comparable amount of fat (including fourteen fatty acids; linoleic acid and oleic acids being the principal unsaturated fatty acids) as well as around 4% of ash, 10% of crude fiber and over 50% of carbohydrates (Al-Jassir, Mustafa, & Nawawy, 1995, Czerwińska & Gulińska, 2012). Aspartic acid, arginine and glutamic acid make up major amino acids. Samh seeds thus have relatively high nutritional value (Doughty, 1936; Palgrave, 1865; Bhattacharya, 1988; Al-Jassir et al., 1995; Al-Qahtani & Maiman, 2011; Alruqaie & Al-Ghamidi, 2015).

Samh flour can be used as a replacement for wheat in 10%, 20% and 30% ratio for bread, and 30%, 60% and 100% ratio for cookies. It improves appearance of cookies, especially their color (Mustafa, Al-Jassir, Nawawy, & Ahmed, 1995; Elgasim & Al-Wesali, 2000). Nutritional studies showed that samh seeds can partly replace corn used for poultry feeding (Najib, Al-Dosari, & AL-Wesali, 2004). Furthermore, it was demonstrated that a diet of 5% samh seeds can normalize cholesterol, glucose and triglycerides level in blood (Al-Faris, Al-Othman, & Ahmad, 2011). Samh seeds flour exhibited reducing effect on creatinine concentration and improved blood lipids, especially cholesterol, HDL and LDL (Al-Qahiz, 2009).

To date, no information was available on the content of secondary metabolites in samh flour, although several phenolic compounds, including rutin, hyperoside, ferulic acid, betacyanin, and flavonol conjugates were isolated from the genus *Mesembryanthemum* (Falleh

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et al., 2009; Falleh et al., 2011). Therefore, the objective of this work was tentative identification of secondary metabolites in samh flour using HPLC–ESI-MS and investigation of their effect on *in vitro* prevention of oxidative damages to human plasma lipids and proteins. To quantify lipid peroxidation, concentration of thiobarbituric acid reactive substances (TBARS) was measured. The oxidative damages to human plasma proteins were measured by the level of thiol groups. Samh flour action was compared with the effects of commercial extract of *Aronia melanocarpa* berries (Aronox®), which is known for various biological activities, including antioxidative role (Olas et al., 2008; Kędzierska et al., 2009). To understand the variation of phenolic acids conjugated with sulfate, a theoretical calculation was carried out based on the DFT/B3LYP approach, and implemented in the GAUSSIAN09 series of programs (Koch & Holthausen, 2001).

2. Material and methods

2.1. Chemicals

Dimethylsulfoxide (DMSO), 5, 5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), and H_2O_2 were purchased from Sigma (St. Louis, MO., USA). All other reagents were of analytical grade. Stock solution of examined plant extracts were made in 50% DMSO. The final concentration of DMSO in samples was lower than 0.05% and its effects were determined in all experiments.

Stock solutions of *A. melanocarpa* extract (commercial name: Aronox® by Agropharm Ltd., Poland; batch no. 020/2007 k) were made in distilled water at a concentration of 5 mg mL^{-1} and kept frozen until used. The total concentration of phenolic derivatives in Aronox® sample used in this study amounted to 309.6 mg g^{-1} of extract, and included phenolic acids (isomers of chlorogenic acid; 149.2 mg g^{-1} of the extract), anthocyanins (cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-xyloside; 110.7 mg g^{-1} of the extract), and flavonoids (quercetin glycosides; 49.7 mg g^{-1} of the extract) (Olas et al., 2008; Kędzierska et al., 2009). The HPLC separation of the extracts from berries of *A. melanocarpa* has been described previously (Olas et al., 2008; Kędzierska et al., 2009).

2.2. Plant material

Samh seeds (*M. forsskalei* Hochst. ex Boiss, syn. *Opophytum forsskalii* (Hochst. ex Boiss.) N.E. Br.) were obtained from Al-Jouf area (northern part of Saudi Arabia Kingdom). Plant material was identified by Prof Arafa I. Hamed according to Täckholm (1974), and the voucher specimen No. 21 was deposited in the Natural Products Laboratory, Chemistry Department, College of Science and Arts (Al-Rass, Qassim University, Saudi Arabia Kingdom).

2.3. Extraction and fractionation

Seeds (500 g) were washed and dried at room temperature without roasting, then ground into flour in the Brabender Wiley mill using 0.5 mm pore sieve according to Mustafa et al., 1995, and exhaustively extracted with 80% MeOH (1.5 L) by maceration at room temperature for 24 h, three times. The crude extract was concentrated under reduced pressure to a syrupy consistency (40 g). 15 g of the crude extract was dissolved in a small quantity of distilled water and loaded on a water preconditioned short C18 column (6 × 10 cm, LiChroprep_ RP-18, 40–60 μm, Merck). Four fractions (1000 mL each) were collected: 100% H_2O (2 g), 60% MeOH (6 g), 80% MeOH (3 g) and 100% MeOH (2.5 g). Fractions were then subjected to qualitative analyses using thin layer chromatography on 10 × 20 cm sheets coated with silica gel F_{254}. A mixture of acetonitrile, distilled water, chloroform and formic acid (10:1:1:0.5 v/v) was used as the mobile phase. Thin layer chromatography (TLC) test shown that only 60% methanolic fraction contains phenolic compounds and glucosinolates, consequently this fraction was used for further studies.

2.4. High-performance liquid chromatography and ESI-mass spectrometry of phenolic fraction

The infusion was analyzed by the HPLC–ESI-IT-MS system using Thermo LCQ Advantage Max ion trap mass spectrometer. Chromatographic separation was carried out on a Thermo Surveyor HPLC system and Waters Xbridge C_{18} column (2.5 μm, 3 mm × 150 mm; Waters, MA, USA). Extract components were separated using a 45 min long linear gradient from 5 to 30% of mobile phase B (acetonitrile containing 0.1% (v/v) formic acid) in mobile phase A (HPLC-grade water containing 0.1% formic acid). The flow rate was 0.3 mL min^{-1}, and the column was held at 50 °C.

The flow from chromatography system was introduced into the ESI ion source operating in the negative ion mode with the following parameters: capillary voltage – 47 V, spray voltage 3.9 kV, tube lens offset – 50 V, capillary temperature 230 °C, sheath gas (N_2) flow rate 70 (arbitrary units), auxiliary gas (N_2) flow rate 10 (arbitrary units). The scan range was *m/z* 150–2000, the maximum injection time was 150 ms with three microscans. Two scan events were arranged to run sequentially in the LCQ mass spectrometer. The first event was a full-scan mass spectrum to acquire data on anions in the designated scan range. The second scan event was an MS/MS experiment at normalized collision energy of 35%, performed on the most prominent $[M-H]^-$ ion acquired during the previous scan event (Rodrigues et al., 2007; Sannomiya et al., 2007; Hamed et al., 2014).

2.5. Plasma isolation

Fresh human plasma was obtained from medication free, regular donors at the blood bank (Lodz, Poland). Plasma was incubated with:

- 60% MeOH fraction from methanolic extract of samh flour at final concentrations of 0.5–50.0 μg mL^{-1} (0, 15, 30 and 60 min, at 37 °C)
- 60% MeOH fraction from methanolic extract of samh flour at final concentrations of 0.5–50.0 μg mL^{-1} plus 2 mM H_2O_2 (0, 15, 30 and 60 min, at 37 °C)
- *A. melanocarpa* extract at a final concentration of 5.0 μg mL^{-1} (15 min, at 37 °C)
- *A. melanocarpa* extract at a final concentration of 5.0 μg mL^{-1} plus 2 mM H_2O_2 (15 min, at 37 °C).

2.6. Lipid peroxidation measurement

Lipid peroxidation was quantified by measuring the concentration of thiobarbituric acid reactive substances (TBARS). Incubation of the plasma (control, plant extract, and 2 mM H_2O_2-treated plasma) was stopped by cooling the samples in an ice-bath. Samples were transferred to an equal volume of 15% (v/v) cold trichloroacetic acid in 0.25 M HCl and 0.37% thiobarbituric acid in 0.25 M HCl; and immersed in a boiling water bath for 15 min. Following centrifugation of samples at 1200 ×*g* for 15 min., the absorbance of clear supernatants at 535 nm (Spectrophotometer UV/Vis Helios alpha Unicam) was measured (Wachowicz, 1984; Rice-Evans, Diplock, & Symons, 1991). The TBARS concentration was calculated using a molar extinction coefficient ($\varepsilon = 156{,}000\ M^{-1}\ cm^{-1}$).

2.7. Thiol groups determination

The thiol group content was measured spectrophotometrically (the absorbance at 412 nm; Spectrophotometer UV/Vis Helios alpha Unicam) with Ellman's reagent — 5,5′-dithio-bis-(2-nitrobenzoic acid). The thiol group concentration was calculated using a molar