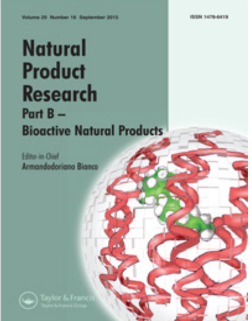

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SHORT COMMUNICATION

Bioassay-guided fractionation of a hepatoprotective and antioxidant extract of pea by-product

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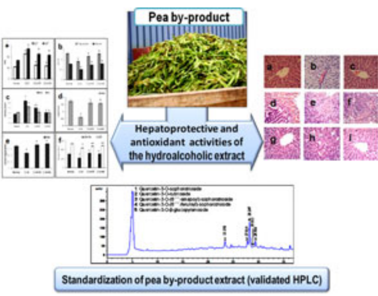

The hepatoprotective and antioxidant activities of the hydroalcoholic extract (PE) of pea (*Pisum sativum* L.) by-product were evaluated, using CCl4-induced oxidative stress and hepatic damage in rats. These activities were assessed via measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein and albumin, malondialdehyde (MDA), reduced glutathione (GSH), protein thiols (PSH), nitrite/nitrate levels, glutathione-peroxidase (GSH-Px), glutathione-S-transferase (GST) activities, as well as, histopathological evaluation. PE revealed significant hepatoprotective and antioxidant activities mostly found in n-butanol fraction. Chromatographic fractionation of this active fraction led to the isolation of five flavonoid glycosides *namely*, quercetin-3-*O*-sophorotrioside (**1**), quercetin-3-*O*-rutinoside (**2**), quercetin-3-*O*-(6''''-*O*-*E* sinapoyl)-sophorotrioside (**3**), quercetin-3-*O*-(6''''-*O*-*E* feruloyl)-sophorotrioside (**4**) and quercetin-3-*O*-β-D-glucopyranoside (**5**). The isolated compounds were quantified in PE, using a validated HPLC method and the nutritional composition of pea by-product was also investigated. Our results suggest that pea by-product contained biologically active constituents which can be utilised to obtain high value added products for nutraceutical use.

Keywords: antioxidant; flavonoids; hepatoprotective; validated HPLC; pea by-product

1. Introduction

Food-processing industries, dealing with vegetables, produce large volume of wastes, which pose increasing disposal and potential severe pollution problems and represent a loss of valuable

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biomass and nutrients (Torres et al. 2003). In Egypt, every year, about 25,000–30,000 tons of peas are processed into frozen peas, yielding large amounts of wastes which represent about 60% of the processed material, mainly composed of pea shells (husks, peels, pericarps or empty pods). Several studies investigated the suitability of pea-processing wastes as animal feed (Abdelhamid & El-Ayoty 1988; Nagib et al. 2001), for biogas production (Madhukara et al. 1997; Ulusoy et al. 2009) or microbial production for the growth of microorganisms, producing either important enzymes or secondary metabolites (Gulati 1987; Verma et al. 2011) and also as a source of dietary fibre (Mateos-Aparicio et al. 2010).

Recent studies reported antihypercholesterolaemic and hypoglycaemic activities, as well as, bifidogenic properties for pea by-product (Khattab & Abdel Wahab 2005; Iwata et al. 2009; Gupta & Premavalli 2011). One report was traced concerning the antioxidant activity of pea peels, using *in vitro* experiments (Dixit & Kar 2009) and two reports dealt with the phenolic constituents of pea by-product (Schmidtlein & Hermann 1975; Winter & Hermann 1986).

To our knowledge, the hepatoprotective activity of this by-product, as well as its active principles, have yet to be studied. Therefore, the aim of this work is to investigate the hepatoprotective and antioxidant activities of pea-processing by-product and to characterise and quantify the active principles responsible for these activities.

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2. Results and discussion

2.1. *Hepatoprotective and antioxidant activities*

The enhanced activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST; Figure S1(a)), as well as, the significant reduction of serum total protein and albumin (Figure S1(b)), on CCl_4 administration, were significantly inhibited on pretreatment with the pea by-product extract (PE), being comparable to the reference drug [Finzelberg standardised cynara extract (NE)]. However, serum ALP activities remained unchanged on CCl_4 administration (data not shown). Increased oxidative stress observed in CCl_4-treated rats was evidenced by remarkable increase in hepatic malondialdehyde (MDA) and NO levels (Figure S1(c)), significant depletion of reduced glutathione (GSH; Figure S1(d)) and protein thiols (PSH) levels (Figure S1(e)), as well as decrease in the activities of glutathione-peroxidase (GSH-Px) and glutathione-*S*-transferase (GST; FigureS1(f)). Pretreatment with the tested extract (PE; Figure S1(c–f)) significantly decreased hepatic MDA and NO, recovered hepatic GSH and PSH levels and restored the activity of GSH-Px and GST towards normal levels being comparable to the normal control group and not significantly different from the reference drug (NE; $P < 0.05$).

To further elucidate whether specific metabolites in pea can account for its hepatoprotective effect, alcohol extract was further subjected to successive fractionation using *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. In the second experiment (Tables S1–S2), the *n*-butanol fraction revealed significant hepatoprotective and antioxidant activities, being equipotent to the reference drug (NE). In addition, the *n*-hexane, methylene chloride, ethyl acetate and aqueous remaining fractions showed no significant effects on the measured biochemical parameters.

2.1.1. *Histopathological examination*

Figure S2(a–i) showed signs due to hepatocellular damage in response to CCl_4 including degeneration, broad infiltration of lymphocytes, vacuolated cytoplasm, dilated sinusoids and pyknotic nuclei in CCl_4 group (Figure S2(b)). Pretreatment with the hydroalcoholic pea extract (PE; Figure S2(c)), as well as the *n*-butanol fraction (Figure S2(g)), resulted in nearly normal hepatic architecture with mild degenerative changes, which was comparable to the reference drug-treated group (Figure S2(i)), meanwhile the other tested fractions did not improve the CCl_4-induced liver damage (Figure S2(d–f&h)). These results confirmed the biochemical data.

Our results are in accordance with previous *in vitro* studies which revealed potent antioxidative potential of the hydroalcoholic extract (50%) of *Pisum sativum* var. *macrocarpon* peels (Dixit & Kar 2009).

Accordingly the significant hepatoprotective and antioxidant effects of the PE were mostly localised in the *n*-butanol fraction. Therefore, this fraction was subjected to further fractionation for isolation of its active constituents.

2.2. *Phytochemical investigation*

Five flavonoidal glycosides (**1**–**5**) were isolated from the active *n*-butanol fraction of pea by-product. Compounds **1**, **3** and **4** (Figure 1) were identified as quercetin-3-*O*-sophorotrioside, quercetin-3-*O*-(6⁗′-*O*-E-sinapoyl)-sophorotrioside and quercetin-3-*O*-(6⁗′-*O*-E-feruloyl)-sophorotrioside, respectively. These compounds were identified based on their UV and NMR (1D [^{1}H and ^{13}C] and 2D [^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC]) spectra (Agrawal 1989; Ferreres et al. 1995; Murakami et al. 2001). The (1 → 2) interglycosidic linkage in the former compounds (**1**, **3** and **4**) was confirmed by HMBC (where long-range correlations were observed between the 1‴-proton and the 2″-carbon and between the 1⁗-proton and the 2‴-carbon (Murakami et al. 2001). In addition, the downfield shift of C-6⁗ (δ_C 63.2, 63.3), together with the upfield shift of C-5⁗ (δ_C 74.3, 74.3) in compounds **3** and **4**, respectively, compared with the non-acylated compound **1** [c.f. C-5⁗ (δ_C 77.8), C-6⁗ (δ_C 61.0) in compound **1**], indicated the site of acylation at C-6 of the terminal glucose moiety (Agrawal 1989, Murakami et al. 2001). Moreover, the downfield shifts of H-6⁗′a (δ_H 4.43, 4.44) and H-6⁗′b (δ_H 4.23, 4.19) in compounds **3** and **4**, respectively, confirmed the acylation at the 6⁗ position of the terminal sugar [c.f. H-6⁗′a (δ_H 3.75) in compound **1**, H-6⁗′b was obscured by the H_2O signal] (Ferreres et al. 1995; Gluchoff-Faisson et al. 1997). Compounds **2** and **5** were identified as rutin and isoquercitrin, respectively, based on their UV data, (Mabry et al. 1970) and cochromatography (TLC and HPLC) with authentic standards. Compounds **1** and **3**–**5** were reported to be isolated from the shoots (Ferreres et al. 1995) and compound **1** from the young seed pods (Murakami et al. 2001) of the plants growing in Spain and Japan, respectively.

Consequently, the antioxidant activities of pea by-product may be attributed to isoquercitrin and rutin (Yang et al. 2008; Silva et al. 2009). Meanwhile, the hepatoprotective activity may be ascribed to querctin-3-*O*-sophortrioside as reported by Murakami et al. (2001). Moreover, the antioxidant effects, as well as the hepatoprotective activities may be due to the presence of acylated flavonols and quercetin-3-*O*-acetylated glycosides (Braca et al. 2003; Lee et al. 2008). Therefore, the hepatoprotective effects of pea extract can be assumed to be related to its antioxidant activity of the isolated flavonoids (Ahmed et al. 2000).

2.3. *Standardisation of the hydroalcoholic extract (PE) using a validated HPLC–UV method*

Five flavonoid glycosides (**1**– **5**) were identified and quantified (mg% ext. dry weight basis) in the extract of pea by-product for its standardisation. Quercetin glycosides (**1**, **2** and **5**) were quantified as rutin (**2**), whereas acylated quercetin glycosides (**3** and **4**) were quantified as quercetin-3-*O*-(6⁗′-*O*-E-feruloyl)-sophorotrioside (**4**). As shown in Figure S3, compound **4** was the principal compound (131.1 mg%), followed by **1** (75.32 mg%), **5** (34.41 mg%) and **3** (27.29 mg%).

2.4. *Nutritive value*

Proximate analysis revealed that the pea by-product contained (DW basis) carbohydrates (61.27%), crude fibre (8%), low fat content (0.79%) and crude protein (14.6%). It contains non-

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Figure 1. Structures of isolated compounds (**1**–**5**).

essential amino acids (7.5%) and essential amino acids (3.95%). In addition, pea by-product was rich in vitamin B_1 (1.61 mg/100 g) and contained vitamins C and B_2 (34.65 & 0.4 mg/100 g, respectively), potassium (25.78%), calcium (8.29%) and magnesium (6.82%).

3. Conclusion

The standardised PE contained health-promoting compounds, namely flavonoids, with significant hepatoprotective and antioxidant activities (Seida et al. 2011), and can be utilised

as functional ingredient to fortify food products, dietary supplement and nutraceutical. However, commercialisation of these by-products for use will need further analysis of pesticide residues and microbial count, to guarantee the product safety, as well as investigations of the stability and interactions of phytochemicals with other food ingredients.

Supplementary material

Experimental details relating to this article are available online, alongside Tables S1–S3 and Figures S1–S3.

References

Abdelhamid AM, El-Ayoty SA. 1988. Feeding sheep on pea by-products produced during preparation for freeze-preservation. J Anim Nutr. 38:789–797.

Agrawal PK. 1989. Carbon-13 NMR of flavonoids. New York: Elsevier.

Ahmed S, Rahman A, Alam A, Saleem M, Athar M, Sultana S. 2000. Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride-induced oxidative stress. J Ethnopharmacol. 69:157–164.

Braca A, Fico G, Morelli I, De Simone F, Tomè F, De Tommasi N. 2003. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. J Ethnopharmacol. 86:63–67.

Dixit Y, Kar A. 2009. Antioxidative activity of some vegetable peels determined *in vitro* by inducing lipid peroxidation. Food Res Int. 42:1351–1354.

Ferreres F, Esteban E, Carpena-Ruiz R, Jiménez MA, Tomás-Barberán FA. 1995. Acylated flavonol sophorotriosides from pea shoots. Phytochemical. 39(6):1443–1446.

Gluchoff-Fiasson K, Fiasson JL, Waton H. 1997. Quercetin glycosides from European aquatic *Ranunculus* species of subgenus *Batrachium*. Phytochemical. 45:1063–1067.

Gulati SL. 1987. Sugars produced from cellulosic wastes as possible substrates for growth of *Rhizobium inocula*. Biol Waste. 21:301–305.

Gupta GP, Premavalli KS. 2011. *In vitro* studies on functional properties of selected natural dietary fibers. Int J Food Prop. 14:397–410.

Iwata E, Hotta H, Goto M. 2009. The screening method of a bifidogenic dietary fiber extracted from inedible parts of vegetables. J Nutr Sci Vitamino. 55:385–388.

Khattab HAH, Abdel Wahab HMF. 2005. Effect of inclusion pea or broad bean husks in diet of hypercholesterolemic rats. Sci Med J (ESCME). 17:17–33.

Lee VS, Chen CR, Liao YW, Tzen JT, Chang CI. 2008. Structural determination and DPPH radical-scavenging activity of two acylated flavonoid tetraglycosides in oolong tea (*Camellia sinensis*). Chem Pharm Bull. 56:851–853.

Mabry JT, Markham KR, Thomas MB. 1970. The systematic identification of flavonoids. New York: Springer-Verlag.

Madhukara K, Srilatha HR, Srinath K, Bharathi K, Krishna N. 1997. Production of methane from green pea shells in floating dome digesters. Process Biochem. 32:509–513.

Mateos-Aparicio I, Redondo-Cuenca A, Villanueva-Suárez MJ. 2010. Isolation and characterisation of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod. Food Chem. 122:339–345.

Murakami T, Kohno K, Ninomiya K, Mastuda H, Yoshikawa M. 2001. Medicinal foodstuffs. XXV. Hepatoprotective principle and structures of ionone glucoside, phenethyl glycoside, and flavonol oligoglycosides from young seedpods of garden peas, *Pisum sativum* L. Chem Pharm Bull. 49:1003–1008.

Nagib AM, Mekkawy SH, Abdel-Fatah OM, Hamza RG. 2001. Effect of using irradiated pea wastes in broiler diets, supplemented with enzyme preparation, on growth performance and digestability. Egypt Poult Sci. 21:883–900.

Schmidtlein H, Herrmann K. 1975. On the phenolic acids of vegetables. II. Hydroxycinnamic acids and hydroxybenzoic acids of fruit and seed vegetables. Z Lebensm Unters Forsch. 159:213–218. [Through PubMed, PMID: 1224795].

Seida AA, El Tanbouly ND, Islam WT, Eid HH, El Maraghy SA, El Senousy AS. 2011. Bioassay-guided fractionation of a hepatoprotective and antioxidant extract of pea by-product. Planta Med. 77, 59th International Congress of the GA, 4–9th September, Antalya, Turkey, 1398, PL106.

Silva CG, Raulino RJ, Cerqueira DM, Mannarino SC, Pereira MD, Panek AD, Silva JF, Menezes FS, Eleutherio EC. 2009. *In vitro* and *in vivo* determination of antioxidant activity and mode of action of isoquercitrin and *Hyptis fasciculata*. Phytomedicine. 16:761–767.

Torres JL, Selga A, Cascante M. 2003. Bioactive products from by-products and wastes. Electron J Environ Agric Food Chem. 2:211–214.

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Ulusoy Y, Ulukardesler AH, Unal H, Alibas K. 2009. Analysis of biogas production in Turkey utilising three different materials and two scenarios. Afr J Agric Res. 4:996–1003.

Verma N, Bansal MC, Kumar V. 2011. Pea peel waste: a lignocellulosic waste and its utility in cellulase production by *Trichoderma reseei* under solid state cultivation. BioResources. 6:1505–1519.

Winter M, Hermann K. 1986. Esters and glucosides of hydroxycinnamic acids in vegetables. J Agric Food Chem. 34:616–620.

Yang J, Guo J, Yuan J. 2008. *In vitro* antioxidant properties of rutin. J Food Sci Technol. 41:1060–1066.

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