Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 498–503
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant and cytoprotective activity of leaves of *Peltiphyllum peltatum* (Torr.) Engl.

Solomon Habtemariam [a,*], Caroline Jackson [b]

[a] *Pharmacognosy and Phytotherapy Research Labs, Medway School of Science, The University of Greenwich, Central Avenue, Chatham-Maritime, Kent ME4 4TN, United Kingdom*
[b] *Hadlow Agricultural College, Hadlow, Tonbridge, Kent TN11 OEX, United Kingdom*

Received 23 October 2006; received in revised form 29 January 2007; accepted 30 March 2007

Abstract

The antioxidant potential of fresh leaves of *Peltiphyllum peltatum* (Torr.) Engl. (Saxfragaceae) was analysed by measuring scavenging potential against 1,1′-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) and hydroxyl radicals (OH$^{\cdot}$), reducing power, inhibition of lipid peroxidation and protection of cultured cells from a lethal dose of hydrogen peroxide (H_2O_2). In all chemical assays used, the crude ethanolic extract of leaves of *P. peltatum*, which contained 21.8 ± 1.7% (w/w, $n = 3$) of total phenols, was as effective as the standard antioxidant compound, rutin. Fractionation of the crude extract with solvent of increasing polarity (namely, petroleum ether, chloroform, ethyl acetate, butanol and water) led to identification of the active fractions (ethyl acetate and butanol fractions). The crude extract and its active fractions, but not rutin, protected cultured RAW 264.7 macrophages from a lethal dose of H_2O_2.

Keywords: *Peltiphyllum peltatum*; Saxifragaceae; Antioxidant; Radical scavenging; Reducing power; Phenol content; Cytoprotection; DPPH; Hydroxyl radical

1. Introduction

Excessive levels of reactive oxygen species, generated either by normal physiological processes or exogenous factors, are known to induce oxidative damage to biological macromolecules such as membrane lipids, proteins and DNA. Such oxidative macromolecular and cellular damage are now known to eventually lead to the development of a variety of disease conditions, including cancer, neurodegenerative diseases, aging and inflammation (Cross et al., 1987; Djordjevi, 2004; Emerit, Edeas, & Bricaire, 2004; Halliwell, Gutteridge, & Cross, 1992; Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). Since foods rich in antioxidant compounds have been shown to reduce the risk of these disease conditions (Kumar & Chattopadhyay, 2007, and references cited therein), numerous recent studies have paid special attention to the search for natural antioxidants, mainly phenolic compounds, for use as dietary supplements, food preservatives and medicine.

Peltiphyllum peltatum (Torr.) Engl. Synonyms – *Darmera peltata* (Torr.) Voss; *Saxifraga peltata* (Torr. ex Benth.) is a perennial plant belonging to the family Saxifragaceae (Stacy, 1992). The plant has distinctive ornamental features, including leafstalks up to 6 ft. tall emerging in spring from an underground stem (rhizome) and dramatic peltate leaf blades up to 2 ft. across. Though the plant is known to be native to the western USA, it is now known to be widely distributed all over Britain and Ireland, colonizing damp places and mainly stream banks (Stacy, 1992). In the western USA, where the plant is known, by its local name as Indian Rhubarb or Umbrella Plant (USDA, 2006), the fleshy edible leafstalks are peeled and eaten raw or put into a salad, cooked like asparagus or added to soups and stews (PFAF, 2006; Usher, 1974). To the best

* Corresponding author. Tel.: +44 (0)208 331 8302; fax: +44 (0)208 331 9805.
E-mail address: S.Habtemariam@gre.ac.uk (S. Habtemariam).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.03.073

of the author's knowledge, neither the chemistry nor the antioxidant activity of *P. peltatum* has previously been reported. The primary aim of the present study was to examine the antioxidant potential of the leaves of *P. peltatum* through a variety of in vitro antioxidant assays.

2. Materials and methods

2.1. Materials

Alamar Blue™ was a product of Serotec (Oxford, UK). Ascorbic acid, β-carotene, 2-deoxy-D-ribose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dulbecco's phosphate buffer saline, ethylenediaminetetraacetic acid (EDTA), $FeCl_2$, $FeCl_3$, gallate, Folin–Ciocalteu reagent, hydrogen peroxide (H_2O_2), linoleic acid, nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), rutin, sodium carbonate, thiobarbituric acid (TBA), tissue culture media and supplements (RPMI medium, heat-inactivated foetal bovine serum, penicillin-streptomycin) and trichloroacetic acid (TCA) were obtained from Sigma–Aldrich Chemical Company (Dorset, UK). All solvents used, namely absolute alcohol, chloroform ($CHCl_3$), ethyl acetate (EtOAc) and butanol, were obtained from Fisher Scientific UK (Leicestershire, UK).

2.2. Extraction of the plant material

Peltiphyllum peltatum (Torr.) Engl., grown for several years around a small stream bank at Broadview Gardens (Hadlow College), was used for the experiment. The leaves were collected during the growing season (July, 2006) and voucher specimens, identified by the Horticulture Group of Hadlow College, were deposited in our laboratory specimen collections for future references. Extraction was carried out by chopping freshly collected leaves (5.8 kg) into small pieces and soaking in 10 l of absolute ethanol for two weeks. Removal of the solvent, using a rotary evaporator, followed by freeze-drying afforded 122 g of the extract residue, hereafter referred to as the CRUDE EXTRACT.

2.3. Fractionation of the crude extract

A portion of the crude extract (40 g) was suspended in water (500 ml) and successively re-extracted with 1 l each (3 times) of petroleum ether (yield: 3 g), chloroform (yield: 1 g), ethyl acetate (yield: 12 g) and finally *n*-butanol (yield: 3 g). All fractions, including the final water fraction (yield: 20 g), were concentrated under reduced pressure using a rotary evaporator and then freeze dried.

2.4. Scavenging of DPPH radical

The method of Blois (1958) was adopted for 96-well plate microtitre assay. Briefly, DPPH$^{\cdot}$ solution (0.1 mM, in methanol) was incubated with varying concentrations of test compounds made in threefold dilutions. The reaction mixture was incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 550 nm against a blank using Multiscan EX Reader (Thermo Labsystems, Altrincham, UK). The radical-scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\%\ \text{Scavenging effect} = (1 - A_{\text{Sample}}/A_{\text{Control}}) - 100$$

where A_{Control} is the absorbance value of DPPH alone and A_{Sample} is absorbance of DPPH and sample mixture minus sample absorbance alone.

2.5. Total phenolic content determination

The total phenolic content of the crude extract and fractions was determined with the Folin–Ciocalteu reagent, following the modified method of Singleton and Rossi (1965). Briefly, various concentrations of test samples made in deionised water (1 ml) were added to 0.5 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with deionised water). After allowing the mixture to stand at room temperature for 5 min, 1.5 ml of 20% (w/v) sodium carbonate were added. Reaction mixtures were further incubated at room temperature for 2 h, following which absorbance at 765 nm was read, using a Jenway 6305 UV–Vis spectrophotometer (Jenway Ltd., Essex, UK). The standard calibration curve was plotted using gallic acid (0.2–125 μg/ml), from which total phenolic content was expressed as percentage (w/w) gallic acid equivalents of extract or fraction.

2.6. Measurement of reducing power

The reducing power of test agents was quantified by the method described by Yen, Duh, and Chua (2000) with minor modifications. Briefly, 1 ml of the reaction mixture, containing different concentrations of samples in Dulbecco's phosphate buffer saline (pH 7.0), was incubated with potassium ferricyanide (1%, w/v) at 50 °C for 20 min. Following termination of the reaction by TCA solution (10%, w/v) and centrifugation (200*g*, 10 min), the aqueous layer was mixed with ferric chloride (0.1%, w/v). Absorbance of the diluted (in deionised water) samples was then read at 700 nm using a Jenway 6305 UV–Vis spectrophotometer. An increase in absorbance of the reaction mixture suggests a greater reducing power.

2.7. Hydroxyl radical scavenging assay

The method of Halliwell, Gutteridge, and Aruoma (1987) was adopted. The reaction mixture, containing test compounds, was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 μM), EDTA (100 μM) and ascorbic acid (100 μM) in Dulbecco's phosphate buffer saline (pH 7.4) for 60 min at 37 °C. The reaction was terminated by adding 1 ml of TBA (1%, w/v) and 1 ml of TCA (2%, w/v) and then heating the tubes in a boiling

water bath for 15 min. The contents were cooled and absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicates decreased oxidation of deoxyribose. The percentage inhibition of deoxyribose oxidation was calculated as $[1-(T/C)]\times 100$, where C is control, malondialdehyde produced by Fenton reaction alone and T is test, malondialdehyde produced in the presence of test compounds.

2.8. The β-carotene/linoleic acid antioxidant activity

The modified method described by Chatterjee et al. (2007) was used. Briefly, 1 ml of a β-carotene solution in chloroform (2 mg in 10 ml) was pipetted into a round bottom flask containing 40 μl of linoleic acid and 500 μl of Tween 20. After the removal of chloroform using a rotary vacuum evaporator at 45 °C, 100 ml of deionised water were added with vigorous agitation. A 2 ml aliquot of the emulsion was added to each well of a 24-well microtitre plate containing various concentrations of test agents. The absorbance was measured at 470 nm immediately against a blank consisting of the emulsion without β-carotene and after 3 h of incubation at 50 °C. All determinations were carried out in quadruplicate. The antioxidant activity of test agents was evaluated in terms of bleaching of β-carotene using the following formula: $AA = [1-(A_0-A_t)/(A'_0-A'_t)]\times 100$ where A_0 and A'_0 are the absorbances measured at zero time of incubation for the test sample and control, respectively, and A_t and A'_0 are the absorbances measured in the test sample and control, respectively, after incubation for 3 h.

2.9. Cytoprotective assay

RAW 264.7 cells obtained from the European Collection of Cell Culture (Porton Down, UK) were routinely maintained with RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum, 50 IU/ml of penicillin and 50 μg/ml of streptomycin in a humidified atmosphere of 95% air-5% CO_2 at 37 °C. For the cytoprotection assay, cells were seeded in 96-well plates at a density of 50,000 cells per well and allowed to establish by incubating plates for 24 h. Various concentrations of the test agents were then added to cell cultures, followed (5 min) by addition of 1 mM H_2O_2 (final concentration in culture) to cause massive onslaught of oxidative damage. After 3 h of incubation, Alamar Blue™ was added to each well, followed by a further incubation for 3 h. Cell viability were then assessed by using the Labsystem's Fluoroskan Ascent FL Fluorometer (Altrincham, UK) at excitation wavelength of 544 nm and emission at 590 nm. Percent protection was calculated as:

$$\%\ \text{Protection} = [1-(F_C-F_S)/(F_C-F_H)]\times 100$$

where F_C is the fluorescent intensity of untreated control groups, F_H is the fluorescent intensity of H_2O_2 treatment alone, and F_S is the fluorescent intensity of H_2O_2 and sample treatment.

2.10. Statistical analysis

Mean and SEM values were calculated from the data. Unpaired student's t-test was used for comparison of results.

3. Results and discussion

3.1. DPPH radical scavenging effect of the crude extract and its fractions

Various concentrations of the crude ethanolic extract of *P. peltatum* leaves and its fractions were first tested for their antioxidant potential in the DPPH assay by using the known plant antioxidant, rutin (Choi et al., 2002; Luis, Valdés, Martín, Carmona, & Díaz, 2006; Tsimogiannis & Oreopoulou, 2006) as a positive control. As shown in Fig. 1, the crude extract showed a concentration-dependent potent DPPH·-radical scavenging effect, with an IC_{50} value of 5.75 ± 0.48 ($n=4$) μg/ml. Surprisingly, this observed scavenging activity was comparable with rutin (IC_{50} value, 5.38 ± 0.56 μg/ml, $n=4$) as there was no significant difference ($p>0.05$) between the two groups (see Fig. 1).

Preliminary fractionation of the active crude extract was done with solvents of increasing polarity: petroleum ether, chloroform, EtOAc, butanol and water. The DPPH-based radical-scavenging assay revealed that the EtOAc (IC_{50} value, 1.73 ± 0.05 μg/ml, $n=4$) and butanol (IC_{50} value, 1.9 ± 0.04 μg/ml, $n=4$) fractions were the most active fractions, with activities about 3 times more potent than the crude extract and/or the standard, rutin (Fig. 1). There appeared to be no significant (unpaired t-test, $p>0.05$) difference between the radical-scavenging effects of the EtOAc and butanol fractions. In contrast to these two active fractions, the petroleum ether, chloroform and water fractions were, by order of magnitudes, weaker than the crude extract (Fig. 1). The overall order of potency was:

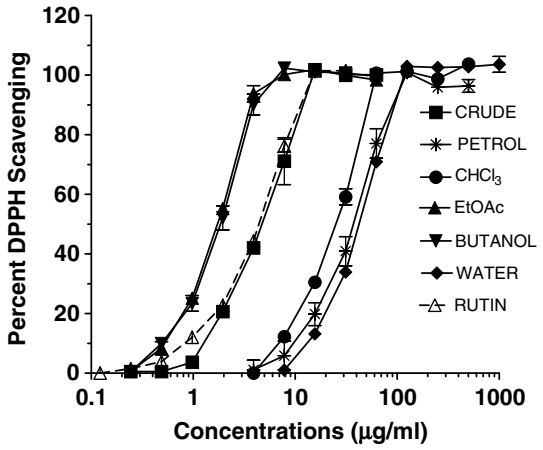

Fig. 1. DPPH·-scavenging activity of ethanolic extract of *P. peltatum* and its fractions. The percent scavenging activity of test compounds obtained from a typical representative experiment is shown. Data are mean values ± SEM ($n=6$).

EtOAc = butanol ≫ crude extract = rutin > chloroform > petroleum ether = water fraction.

3.2. Total phenol content of the crude extract and its fractions

Since the crude extract and its fraction displayed potent radical-scavenging activity, and to date no report exists on the chemical constituents of the plant, the total phenolic contents of all samples were determined. As shown by the gallate equivalent assay, the crude extract appeared to be packed full of phenolics with mean percentage (w/w) phenolic content of 21.8 ± 1.66 ($n = 3$). The petroleum ether, chloroform and water fractions which showed weaker radical scavenging effects than did the crude extract, appeared to have lower total phenol contents: 1.9 ± 0.58, 5.6 ± 1.37 and 2.0 ± 0.22 ($n = 3$), respectively. The EtOAc and butanol fractions, which displayed the highest DPPH radical-scavenging effect, showed the highest total phenol content: 44.9 ± 2.14 and 40.1 ± 1.66 ($n = 3$), respectively. Thus, the order (highest first) of total phenol content was similar to the observed DPPH scavenging effect: i.e., EtOAc = butanol > crude extract > chloroform ≫ petroleum ether = water fraction.

In order to further establish the relationship between total phenol content and DPPH radical scavenging activity of crude extract and fractions, a correlation graph was plotted (Fig. 2). A positive correlation, clearly showing an increase in radical scavenging activity (decreased IC_{50} value) with increasing total phenol content, was established (Fig. 2). A straight line (correlation coefficient $r^2 = 0.85159$) could not, however, be plotted from this data, suggesting that there is no direct linear relationship between total phenolic content and DPPH·-scavenging activities. Since a mixture of several constituents is involved in producing the antioxidant effects of plant extracts and/or fractions, this non-linear relationship is expected and was also in agreement with similar previous correlation studies (Anagnostopoulou, Kefalas, Papageorgiou, Assimopoulou, & Boskou, 2006).

3.3. Reducing power of the crude extract and its fractions

The antioxidant effect of many compounds have been shown to be related to the presence of reductones which exert antioxidant action by breaking the free radical chain through donation of a hydrogen atom (Qi et al., 2005). The reducing capacity of a compound could thus further serve as a significant indicator of potential antioxidant activity. In the present study, the reductive capacities of the crude extract and its various fractions were determined, based on measurement of Fe^{3+}–Fe^{2+} transformation. The concentration-dependent reducing power effect shown in Fig. 3 suggests that the crude extract and its active fractions, as electron donors, can react with free radicals to convert them to more stable products, leading to termination of radical chain reactions. It is interesting to note that the orders of potency, both for the positive control, rutin and test extract/fractions, are similar to the order of DPPH-scavenging effect (Fig. 3).

3.4. Hydroxyl radical scavenging activity

OH· is by far the most reactive free radical species known to date, with damaging effect to almost every biological molecule found in living cells. The OH has been shown to be readily formed from $O_2^{\cdot-}$ radicals in vivo through transition metal (e.g. iron or copper)-catalysed Haber–Weiss reaction (Castro & Freeman, 2001). A further test, utilising the OH generation system, is thus vital in order to validate

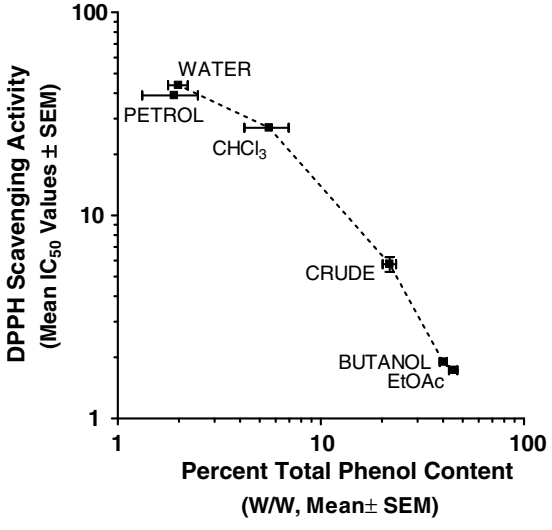

Fig. 2. Relationship between total phenol content and DPPH-scavenging activity. Mean total phenol contents and IC_{50} values for DPPH-scavenging activity obtained from four separate experiments are shown for the crude ethanolic extract and its fractions.

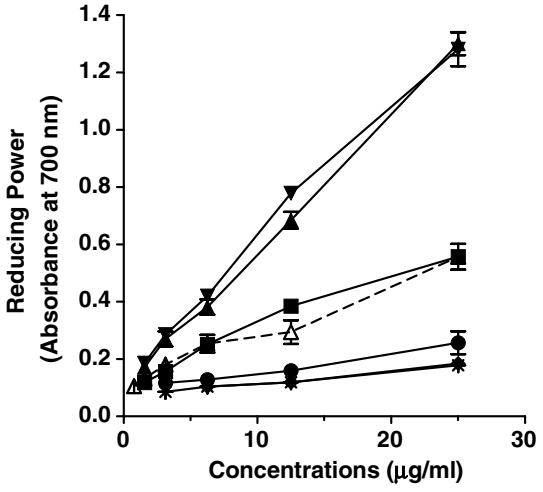

Fig. 3. Reducing power of ethanolic extract of *P. peltatum* and its fractions based on measurement of Fe^{3+}–Fe^{2+} transformation. Data points are mean values ± SEM ($n = 4$).

the radical-scavenging effect and/or antioxidant potential of test compounds. In the present study, an in vitro Fenton-type assay system ($Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$) that measures the generation of OH and its subsequent effect on deoxyribose oxidation (Halliwell et al., 1987) was used. The system involves autoxidation of an iron(II)–EDTA complex in aqueous solution to form O_2^-, which is rapidly dismutated to H_2O_2 at pH 7.4. The H_2O_2 further interacts with iron(II), to form OH radicals in the presence of ascorbic acid as a catalyst (Caillet et al., 2007, and references cited therein). Fig. 4 displays the potent concentration-dependent OH·-scavenging effect of the crude extract and its active fractions. As with the DPPH· and reducing power assays, the EtOAc fraction showed the highest OH-scavenging activity ($IC_{50} = 2.5 \pm 0.2$ μg/ml, $n = 3$) and the crude extract ($IC_{50} = 3.6 \pm 0.06$ μg/ml, $n = 3$) displayed an OH·-scavenging activity as potent as rutin ($IC_{50} = 3.33 \pm 0.09$ μg/ml, $n = 3$). The activity profile of the butanol fraction (Fig. 4), however, appeared to be different from the DPPH· and reducing power assays in that it was about twice weaker ($IC_{50} = 8.33 \pm 0.9$ μg/ml, $n = 3$) than was the crude extract. This may be explained by the presence of different constituents in the fractions contributing to variable responses in the various assays. Moreover, the OH· generation by the Fenton reaction could involve an activity other than free radical-scavenging.

3.5. Antioxidant activity in β-carotene/linoleic acid emulsion system

Cell membranes are composed of phospholipid bilayers, with intrinsic/extrinsic proteins, and are direct targets for lipid oxidation by reactive oxygen species. The potential antioxidant property of *P. pelttum* in this model of antioxidant study was measured by using the β-carotene bleaching assay. The result obtained (Fig. 5) was similar to the OH·-scavenging assay in that the butanol fraction ($IC_{50} = 117$ μg/ml $\pm$ 3, $n = 3$) appeared to possess a significantly ($p < 0.01$) weaker effect than the crude extract ($IC_{50} = 81 \pm 2.2$ μg/ml, $n = 3$), rutin ($IC_{50} = 71 \pm 4.9$ μg/ml, $n = 3$) and the EtOAc fraction ($IC_{50} = 59 \pm 1$ μg/ml, $n = 3$). This demonstrated comparable antioxidant activity of the crude extract to the standard, rutin, and the identification of an even more potent active fraction (the EtOAc fraction) suggests that *P. peltatum* has great potential to be exploited as an antioxidant source.

3.6. Cytoprotective effect

H_2O_2-mediated cytototoxicity is the commonest method employed for the measurement of potential cytoprotective antioxidants (Chow, Shen, Huan, Lin, & Chen, 2005; Fallarero, Loikkanen, Männistö, Castañeda, & Vidal, 2003; García-Alonso, Ros, & Periago, 2006; Godkar, Gordon, Ravindran, & Doctor, 2003). Not all antioxidants, however, protect cells from massive oxidative insult by milimolar concentrations of H_2O_2. In this connection and consistent with previous results (Arredondo et al., 2004; Chow et al., 2005), the positive control, rutin, failed to protect cultured macrophages from H_2O_2 cytotoxicity in the present study (Fig. 6). It is worth noting that all tested concentrations of rutin, the crude extract and the EtOAc and butanol fractions were found to have no direct cytotoxic effect on RAW 264.7 macrophages over the 6 h incubation period (data not shown). Unlike the positive control, rutin, the crude extract and its active fractions displayed concentration-dependent protection against H_2O_2 cytotoxicity and the activity profile of the butanol fraction parallels those of the OH-scavenging and lipid peroxidation assays (Fig. 6).

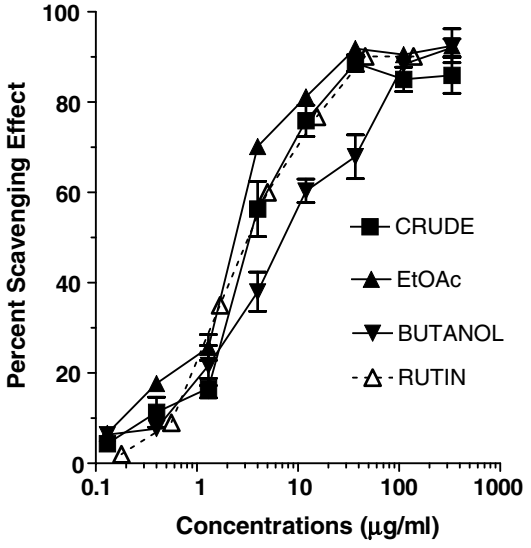

Fig. 4. Hydroxyl radical scavenging effect of crude ethanolic extract of *P. peltatum* and its active fractions. The percentage inhibition of deoxyribose oxidation induced by hydroxyl radical generated from the Fenton reaction is shown. Data are mean values ± SEM ($n = 3$).

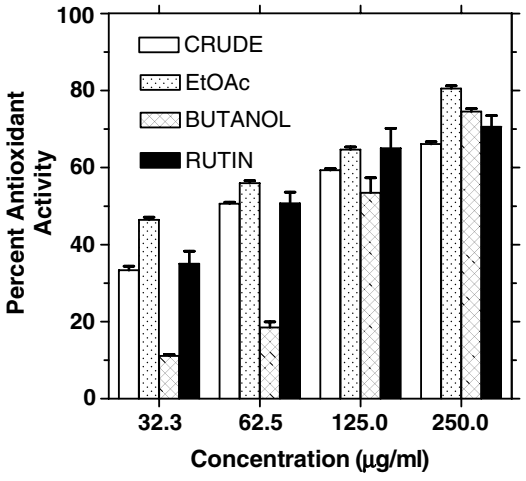

Fig. 5. Antioxidant activity of ethanolic extract of leaves of *P. peltatum* and its active fractions in the β-carotene/linoleic acid system. Values are means of triplicate determinations ± SEM.

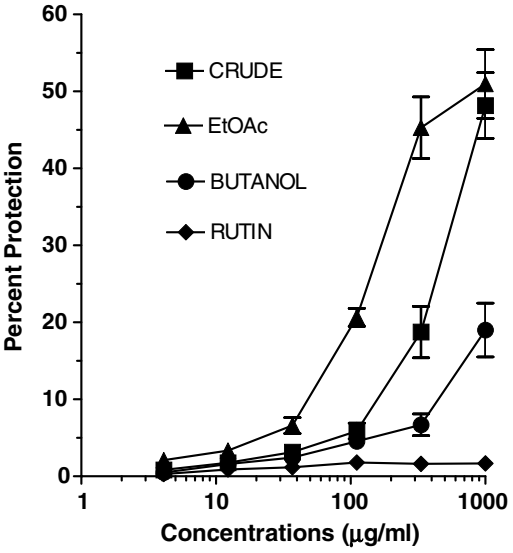

Fig. 6. Cytoprotective activity of crude ethanolic extract of leaves of *P. peltatum* and its active fractions. Experiments were carried out as described in the materials and methods. Data points are means ± SEM ($n = 4$).

4. Conclusions

The present study clearly demonstrated that an ethanolic extract of leaves of *P. peltatum* exhibits potent radical-scavenging, reducing power and antioxidant activity. The antioxidant results in the chemical assays were further validated by the demonstration that the crude extract protected cultured macrophages from H_2O_2-induced cytotoxicity. An activity-directed fraction of the crude ethanolic extract led to the identification of the EtOAc fraction as the most radical scavenging, antioxidant and cytoprotective fraction. *P. peltatum* has thus a great potential to be exploited as a natural source of antioxidants. Further work is now being carried out to isolate and characterise the active antioxidant principles.

References

Anagnostopoulou, M. A., Kefalas, P., Papageorgiou, V. P., Assimopoulou, A. N., & Boskou, D. (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). *Food Chemistry, 94*(1), 19–25.

Arredondo, M. F., Blasina, F., Echeverry, C., Morquio, A., Ferreira, M., Abin-Carriquiry, J. A., et al. (2004). Cytoprotection by *Achyrocline satureioides* (Lam) D.C. and some of its main flavonoids against oxidative stress. *Journal of Ethnopharmacology, 91*(1), 13–20.

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature, 181*, 119–200.

Caillet, S., Yu, H., Lessard, S., Lamoureux, G., Ajdukovic, D., & Lacroix, M. (2007). Fenton reaction applied for screening natural antioxidants. *Food Chemistry, 100*(2), 542–552.

Castro, L., & Freeman, B. A. (2001). Reactive oxygen species in human health and disease. *Nutrition, 170*(2), 161–165.

Chatterjee, S., Niaz, Z., Gautam, S., Adhikari, S., Variyar, P. S., & Sharma, S. (2007). Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*). *Food Chemistry, 101*(2), 515–523.

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., et al. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science, 163*(6), 1161–1168.

Chow, J.-M., Shen, S.-C., Huan, S. K., Lin, H.-Y., & Chen, Y.-C. (2005). Quercetin, but not rutin and quercitrin, prevention of H_2O_2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. *Biochemical Pharmacology, 69*(12), 1839–1851.

Cross, C. E., Halliwell, B., Borisch, E. T., Pryor, W. T., Ames, B. N., Saul, R. L., et al. (1987). Oxygen radicals and human disease. *Annals of Internal Medicine, 107*(4), 526–545.

Djordjevi, V. B. (2004). Free radicals in cell biology. *International Review of Cytology, 237*, 57–89.

Emerit, J., Edeas, M., & Bricaire, F. (2004). Neurodegenerative diseases and oxidative stress. *Biomedecine & Pharmacotherapy, 58*(1), 39–46.

Fallarero, A., Loikkanen, J. J., Männistö, P. T., Castañeda, O., & Vidal, A. (2003). Effects of aqueous extracts of *Halimeda incrassata* (Ellis) Lamouroux and *Bryothamnion triquetrum* (S.G.Gmelim) Howe on hydrogen peroxide and methyl mercury-induced oxidative stress in GT1-7 mouse hypothalamic immortalized cells. *Phytomedicine, 10*(1), 39–47.

García-Alonso, G., Ros, G., & Periago, M. J. (2006). Antiproliferative and cytoprotective activities of a phenolic-rich juice in HepG2 cells. *Food Research International, 39*(9), 982–991.

Godkar, P., Gordon, R. K., Ravindran, A., & Doctor, B. P. (2003). *Celastrus paniculatus* seed water soluble extracts protect cultured rat forebrain neuronal cells from hydrogen peroxide-induced oxidative injury. *Fitoterapia, 74*(7–8), 658–669.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*(1), 215–219.

Halliwell, B., Gutteridge, J. M. C., & Cross, C. E. (1992). Free radicals, antioxidants and human disease: Where are we now? *Journal of Laboratory and Clinical Medicine, 119*(6), 598–620.

Kumar, A., & Chattopadhyay, S. (2007). DNA damage protecting activity and antioxidant potential of pudina extract. *Food Chemistry, 100*(4), 1377–1384.

Luis, J. C., Valdés, F., Martín, R., Carmona, A. J., & Díaz, J. D. (2006). DPPH radical scavenging activity of two flavonol glycosides from *Aconitum napellus* sp. *lusitanicum*. *Fitoterapia, 77*(6), 469–471.

PFAF (Plants for a future). (2006). *Edibile and medicinal plants for a healthier world*. Available from http://www.pfaf.org.

Qi, H., Zhang, Q., Zhao, T., Chen, R., Zhang, H., Niu, X., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. *International Journal of Biological Macromolecules, 37*(4), 195–199.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Stacy, C. A. (1992). *New flora of British Isles*. Suffolk: St. Edmundsbury Press Ltd., p. 385.

Tsimogiannis, D. I., & Oreopoulou, V. (2006). The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3′,4′-hydroxy substituted members. *Innovative Food Science & Emerging Technologies, 7*(1–2), 140–146.

Usher, G. (1974). *A dictionary of plants used by man*. Prescot, Lances: Tinling (1973) Ltd., p. 528.

USDA (United State department of Agriculture). (2006). *Natural resources conservation service – plants database*. Available from Http://plants.usda.gov.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*(1), 1–40.

Yen, G.-C., Duh, P.-D., & Chua, D.-Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chemistry, 70*(4), 437–444.