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Free radical scavenging activity of an aqueous extract of potato peel

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Abstract

The antioxidant potency of freeze-dried aqueous extract of potato peel was investigated employing various established in vitro systems, such as lipid peroxidation in rat liver homogenate, 1, 1-diphenyl-2-picrylhydrazyl (DPPH)/superoxide/hydroxyl radical scavenging, reducing power, and iron ion chelation. Freeze-dried aqueous extract of potato peel powder (PPE) showed strong inhibitory activity toward lipid peroxidation of rat liver homogenate induced by the $FeCl_2$–H_2O_2 system. Furthermore, PPE exhibited a strong concentration-dependent inhibition of deoxyribose oxidation. PPE also showed a considerable antioxidant activity in the DPPH radical assay system. The multiple antioxidant activity of PPE was evident as it showed strong reducing power, superoxide scavenging ability and also ferrous ion chelating potency. The data obtained in the in vitro models clearly establish the antioxidant potency of freeze-dried extract of potato peel. Considering that potato peels are discarded as waste and not effectively utilized, these in vitro results suggest the possibility that potato peel waste could be effectively employed as an ingredient in health or functional food, to alleviate oxidative stress. However, comprehensive studies need to be conducted to ascertain the in vivo safety of such extracts in experimental animal models.
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Keywords: Potato peel extract; Antioxidant activity; Lipid peroxidation; Radical scavenging effect; Iron chelation

1. Introduction

Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases as well as in the normal process of aging (Halliwell, Gutteridge, & Cross, 1992). Reactive oxygen species (ROS) in the forms of superoxide anion ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2) are generated by normal metabolic processes or from exogenous factors and agents, and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. These ROS are capable of damaging a wide range of essential biomolecules (Halliwell & Gutteridge, 1990). Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging ROS, activating a battery of detoxifying proteins, or preventing the generation of ROS (Halliwell et al., 1992). In recent years, there has been increasing interest in finding natural antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993). Natural antioxidants constitute a broad range of compounds including phenolic compounds, nitrogen compounds and carotenoids (Velioglu, Mazza, Gao, & Oomah, 1998). The antioxidant activity of several plant materials has recently been described (Al Saikhan, Howard, & Miller Jr., 1995; Amarowicz, Wanasundhara, Karamac, & Shahidi, 1996; Bergman, Varshavsky, Gottlieb, & Grossman, 2001; Cao, 1996; Oomah & Mazza, 1994; Wang, Cao, & Prior, 1996; Yen & Duh, 1995). The search for newer natural antioxidants, especially of plant origin has ever since increased.

Potato peel, a waste by-product from potato processing, could be considered as a new source of natural antioxidant. Potato peel is found to contain phenolic acids (Lisinska & Leszczynski, 1987) and recently the antioxidant activity of potato peel extract has been studied in food systems (Rodriguez de Sotillo, Hadley, & Holm, 1994b). However, convincing evidence for the free radical scavenging activity of potato peel extract is

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still needed. Hence, the present work investigates the possible antioxidative effects of freeze-dried powder procured from aqueous extract of potato peel. In this study, we studied the antioxidant activity of PPE, employing various in vitro assay systems, such as inhibition of lipid peroxidation, DPPH/superoxide/hydroxyl radical scavenging, and iron ion chelation, in order to understand the mechanisms of its antioxidative activity.

2. Materials and methods

2.1. Chemicals

Butylated hydroxyltoluene (BHT), nitro blue tetrazolium (NBT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), phenazine methosulphate (PMS), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA) and ethylenediamine tetra-acetic acid (EDTA) were purchased from M/s Sigma Chemicals Co. (St.Louis, MO). Ferrozine, nicotinamide adenine dinucleotide-reduced (NADH), trichloroacetic acid (TCA), deoxyribose (DR), potassium ferricyanide and ferric chloride were purchased from M/s Sisco Research Laboratories, Mumbai, India. All other reagents were of analytical grade.

2.2. Preparation of freeze-dried extract of potato peel (PPE)

Fresh potato peels, obtained from local potato chip making units, were washed three times with tap water and then dried at 70 °C for 5 h in a 'cross-flow air drier'. The dried peel was ground in a multimill and passed through a 0.5 mm sieve to obtain a fine powder. Potato peel powder (0.5 g) was homogenized with 10 ml of distilled water for 5 min and the homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through Whatman No.1 filter paper, and the resultant extract was lyophilized to dryness in vacuo. The lyophilized powder (PPE) was stored in a dark bottle at 4 °C until use.

2.3. Inhibition of lipid peroxidation in rat liver homogenate

Rat liver, excised from male Wistar rats (120–125 g), was homogenized (1% w/v) in 0.154 M potassium chloride solution. The homogenate was centrifuged at 3000 rpm at 4 °C for 10 min and the supernatant used for the assay. Peroxidation of the liver homogenate was induced by $FeCl_2$–H_2O_2 (Yen & Hsieh, 1998). Briefly, 1% liver homogenate was incubated with 0.5 mM, each, of $FeCl_2$ and H_2O_2 with or without PPE (0.75–5.0 mg). After incubation at 37 °C for 60 min, the formation of MDA in the incubation mixture was measured at 535 nm (Buege & Aust, 1978). BHT was used as positive control.

2.4. Scavenging of DPPH radical

The effect of PPE on DPPH radical was studied, employing the modified method described earlier by Yamaguchi, Takamura, Matoba, and Terao (1998). Briefly, 1.5 ml of DPPH solution (0.1 mM, in 95% Ethanol) was incubated with varying concentrations of the extract (PPE, 0.75–5.0 mg). The reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_{\text{Sample}(517\ \text{nm})}}{A_{\text{Control}(517\ \text{nm})}}\right) \times 100$$

2.5. Measurement of reducing power

The reducing power of the extract was quantified by the method described earlier by Yen and Chen (1995) with minor modifications. Briefly, 1 ml of reaction mixture, containing PPE (1.25–5.0 mg) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50 °C for 20 min. The reaction was terminated by adding TCA solution (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.6. Superoxide-radical scavenging assay

The superoxide scavenging ability of the extract was assessed by the method of Nishikimi, Rao, and Yagi (1972). The reaction mixture, containing PPE (0.25–5.0 mg), PMS (30 μM), NADH (338 μM) and NBT (72 μM) in phosphate buffer (0.1 M pH 7.4), was incubated at room temperature for 5 min and the colour was read at 560 nm against a blank. The capability of scavenging the superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_{\text{Sample}\ 560\ \text{nm}}}{A_{\text{Control}\ 560\ \text{nm}}}\right) \times 100$$

2.7. Hydroxyl radical assay

The reaction mixture, containing PPE (1.25–5.0mg), 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 potassium phosphate buffer (20 mM, pH7.4) for 60 min at 37 °C (Halliwell, Gutteridge, & Aruoma, 1987). 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 the absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose.

2.8. Metal ion chelating assay

The ferrous ion-chelating potential of the extract was investigated according to the method of Decker and Welch (1990), wherein the Fe^{2+}-chelating ability of the extract was monitored by measuring the ferrous iron-ferrozine complex at 562 nm. Briefly, the reaction mixture, containing PPE (1.25–5.0 mg), $FeCl_2$ (2 mM), and ferrozine (5 mM), was adjusted to a total volume of 0.8 ml with methanol, shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank. EDTA (0.05–0.2mg) was used as positive control. The ability of the extract to chelate ferrous ion was calculated using the following equation:

$$\text{Chelating effect (\%)} = \left(1 - \frac{A_{\text{Sample 562 nm}}}{A_{\text{Control 562 nm}}}\right) \times 100$$

2.9. Statistical analysis

All data are expressed as means±S.D. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test. Results were processed by computer programmes: Excel and Statistica software (1999).

3. Results

3.1. Inhibition of lipid peroxidation by potato peel extract

The endogenous basal levels of MDA in the rat liver homogenate were in the range of 44.1 nmol/g tissue. At the end of 30 min incubation with $FeCl_2$–H_2O_2, the induction of MDA was increased by 10-fold.

While the lowest concentration yielded marginal protection, significant inhibitory response was evident from 1 mg onwards. Although the effect was not concentration-related, maximum protection was evident at the highest concentration (Fig. 1).

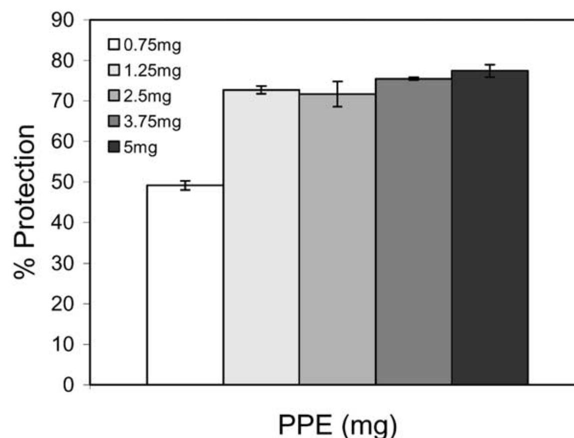

Fig. 1. Inhibition of lipid peroxidation by freeze dried extract of potato peel in rat liver homogenate. Each value represents mean±standard deviation ($n=6$).

3.2. DPPH radical scavenging activity of potato peel

Total DPPH scavenging potential of the freeze-dried extract of potato peel (PPE) at varying concentrations was measured and the results are depicted in Fig. 2.

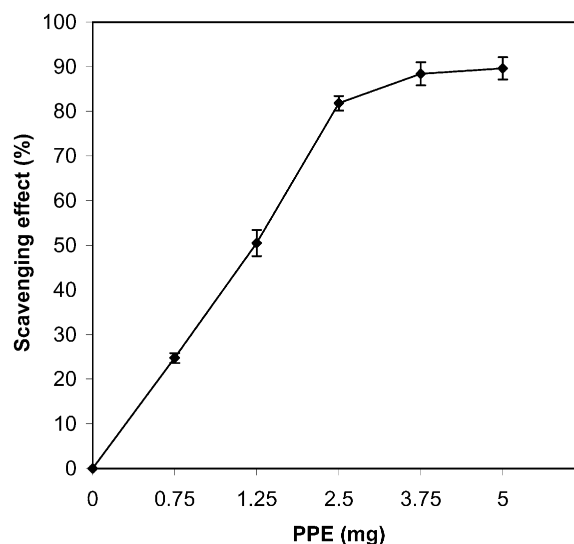

Fig. 2. Scavenging effect of freeze dried extract of potato peel (PPE) on DPPH radical. Values are means±S.D. of three determinations.

Significant DPPH radical scavenging activity was evident at all the tested concentrations of PPE. The scavenging effect increased with increasing PPE concentration up to a certain extent (3.75 mg) and then levelled off with further increase.

3.3. Reducing power of potato peel extract

Fig. 3 depicts the reducing power of both PPE and BHT, a known antioxidant. The reducing power of PPE (as indicated by the absorbance at 700 nm) correlated well with increasing concentrations. However, as anticipated, the reducing power of BHT was relatively more pronounced than that of PPE.

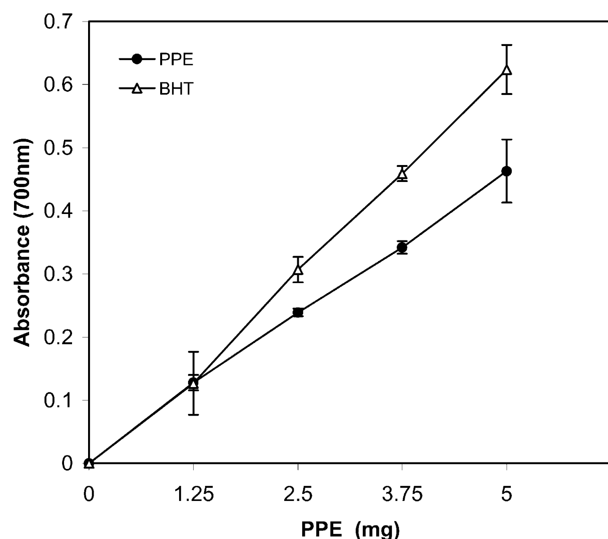

Fig. 3. Reducing power of PPE and BHT (10–200 μM) Values are means S.D. of three determinations; PPE, freeze-dried extract of potato peel; BHT-butylated hydroxytoluene.

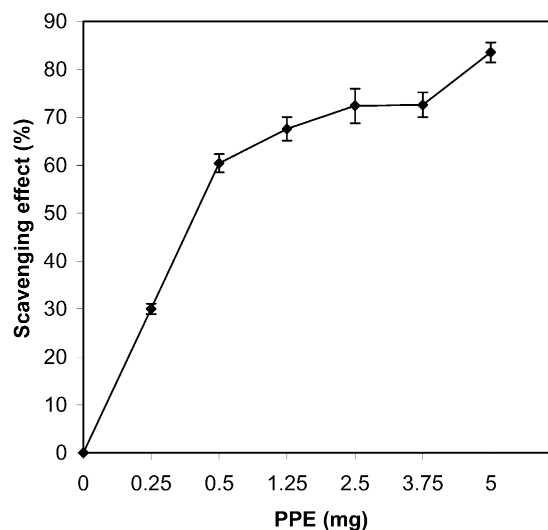

Fig. 4. Scavenging effect of PPE on superoxide radical. Values are means ±SD of three determinations.

3.4. Scavenging of superoxide radical by PPE

Fig. 4 shows that the inhibitory effect of PPE on superoxide radicals was marked and concentration-related. Significant scavenging (60–84%) of superoxide radicals was evident at all the tested concentrations of PPE (0.5–5.0 mg).

3.5. Hydroxyl radical scavenging activity of PPE

The effect of PPE on oxidative damage, induced by Fe^{3+}/H_2O_2 on deoxyribose, as measured by the thiobarbituric acid method, is plotted in Fig. 5. While a marginal inhibition was evident at the lowest concentration, nearly 75% inhibition was observed at the highest concentration.

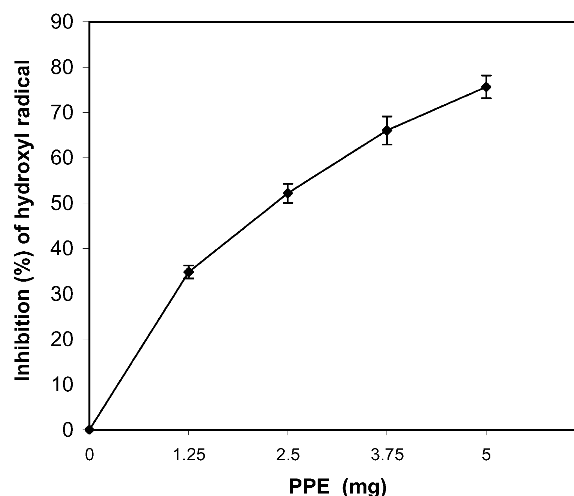

Fig. 5. Inhibitory effect of PPE on deoxyribose oxidative damage. Values are means±S.D. of three determinations.

3.6. Ferrous ion-chelating effect of PPE

The ferrous ion-chelating effect of PPE was concentration related as shown in Fig. 6. A maximum effect (50%) was evident at a concentration of 5 mg PPE, which was comparable to that of the chelating activity of EDTA at the lowest concentration of 0.05 mg.

4. Discussion

The antioxidative activity of polyphenolic compounds present in potato has been demonstrated earlier in several food systems (Onyeneho & Hettiarachchy, 1993; Rodriguez de Sotillo et al., 1994a, 1994b). However, the ability of aqueous extract of potato peel to scavenge free radicals in chemical and biological systems has not been comprehensively investigated. Accordingly, this study primarily aims to elucidate the antioxidant attributes of freeze-dried extract of potato peel (PPE), employing a wide range of well established *in vitro* systems to gain mechanistic insights.

In biological systems, lipid peroxidation (oxidative degradation of polyunsaturated fatty acid in the cell membranes) generates a number of degradation products, such as malondialdehyde (MDA), and is found to be an important cause of cell membrane destruction and cell damage (Yoshikawa, Naito, & Kondo, 1997). MDA, one of the major products of lipid peroxidation, has been extensively studied and measured as an index of lipid peroxidation and as a marker of oxidative stress (Janero, 1990). In the present study, we measured the potential of PPE to inhibit lipid peroxidation in rat liver homogenate, induced by the $FeCl_2$–H_2O_2 system. The hydroxyl radical, known to be generated through the

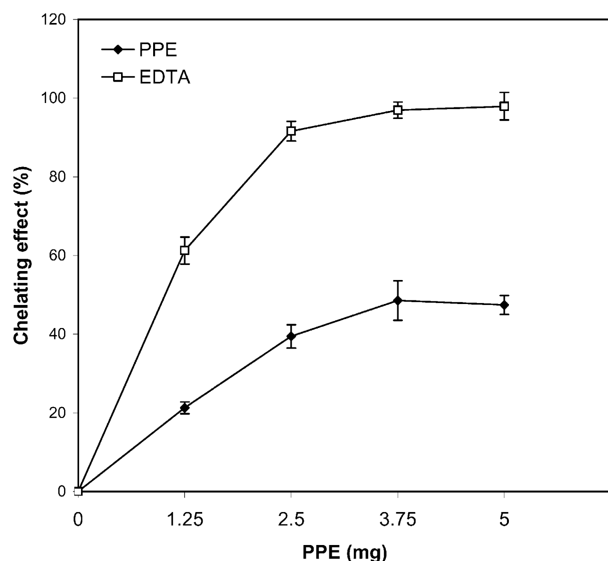

Fig. 6. Chelating effect of PPE and EDTA (0.05–0.2 mg) on Fe^{2+} ion. Values are means ± S.D. of three determinations.

Fenton reaction in this system, was scavenged significantly by co-incubation of rat liver homogenate with varying concentrations of PPE. Further evidence of the hydroxyl radical scavenging activity of PPE was obtained in the deoxyribose system. In this system, PPE exhibited a stronger concentration-dependent inhibition of deoxyribose oxidation. Earlier, numerous workers (Halliwell, Gutteridge, & Aruoma, 1987; Pin-Der-Duh, 1998) have employed this system to assess the biological activity of various natural plant derived biomolecules. Smith, Halliwell, and Aruoma (1992) earlier reported that molecules that can inhibit deoxyribose degradation are those that can chelate iron ions and render them inactive or poorly active in a Fenton reaction. In the present study, in another assay system, we demonstrated the iron chelating ability of the PPE. It is likely that the chelating effect of PPE on metal ions may be responsible for the inhibition of deoxyribose oxidation. Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases (Halliwell & Gutteridge, 1990). Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation, and reduction of Fe^{2+} concentrations in the Fenton reaction would protect against oxidative damage.

The antioxidant activity of PPE was also discernible in the DPPH radical assay, which primarily evaluates proton radical-scavenging ability. DPPH is one of the compounds that possesses a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Yamaguchi, Takamura, Matoba, & Terao, 1998). Further it is well accepted that the DPPH free radical-scavenging by antioxidants is due to their hydrogen-donating ability (Chen & Ho, 1995). In the present study, PPE showed a concentration dependent scavenging of DPPH radical, which may be attributable to its hydrogen-donating ability.

Earlier authors (Pin-Der Duh, Pin-Chan-Du & Gow-Chin Yen, 1999; Tanaka, Kuie, Nagashima, & Taguchi, 1988; Pin-Der Duh, 1998) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Pin-Der Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of PPE suggest that it is likely to contribute significantly towards the observed antioxidant effect.

Although superoxide is a relatively weak oxidant, it decomposes to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids (Dahl & Richardson, 1978). In the present study, PPE effectively scavenged superoxide in a concentration-dependent manner. Further, superoxides are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Meyer & Isaksen, 1995). These results clearly suggest that the antioxidant activity of PPE is also related to its ability to scavenge superoxides.

In conclusion, the results obtained in the present study clearly demonstrate that the aqueous extract of potato peel contains a number of antioxidant compounds, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. The broad range of activity of the extract suggests that multiple mechanisms are responsible for the antioxidant activity of PPE. Although we have not isolated the compounds responsible for the antioxidant activity, we speculate that it may be related to the phenolic acids in the extract. The qualitative/quantitative analysis of the extracts for phenolic acids showed the presence of chlorogenic acid, gallic acid, caffeic acid and protocatechuic acids (Nandita Singh & Rajini 1999; Nandita Singh, 2002) which is consistent with the earlier reports (Rodriguez de Sotillo et al., 1994a,1994b; Rodriguez de Sotillo, Hadley, & Wolf-Hall, 1998). The multiple antioxidant activity of freeze dried extract potato peel demonstrated in this study clearly indicates the potential application value of the potato peel. However, the in vivo safety of potato peel *per se* needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods

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