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Antioxidant properties of different fractions of tubers from *Pueraria tuberosa* Linn

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Abstract

Pueraria tuberosa Linn. (PT), *Leguminosae*, is a perennial climber, growing throughout tropical parts of India. In the Ayurvedic system of medicine, it is used as a drug of choice to manage pain, inflammation and other related diseases. The antioxidant potency of *P. tuberosa* was investigated for the first time. Total antioxidant capacity was determined using an ABTS*+ assay. Lipid peroxidation was assessed in terms of thiobarbituric acid-reactive substances by using egg-yolk homogenates as lipid-rich media. Superoxide radical-scavenging was measured using riboflavin-light-nitro blue tetrazolium (NBT) assay. Hydroxyl radical trapping potential was determined by evaluating hydroxyl radical induced deoxyribose degradation using thiobarbituric acid method. In order to assess the metal chelation property, hydroxyl radical induced deoxyribose degradation was evaluated in the absence of ethylenediaminetetraacetic acid. Both hexane and methanol fractions inhibited lipid peroxidation and also chelated the iron, showing potent antioxidant property.

Keywords: *Pueraria tuberosa*; Antioxidants; Metal chelation; Inflammation

1. Introduction

Pueraria tuberosa Linn, *Leguminosae*, known as Bida-arikand, is an extensive perennial climber, growing throughout tropical parts of India, mostly in moist regions, monsoon-forests and coastal tracts (Chopra, Nayar, & Chopra, 1956). It has palmate leaves with blue-coloured flowers and half-inch thick bark (Pandey & Chunekar, 1998). Its roots and leaves have an extensive range of medicinal properties.

Here, we have studied the antioxidant capacity of two fractions of *P. tuberosa*, by employing various established *in vitro* assay systems.

2. Materials and methods

2,2′-Azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) and deoxyribose were purchased from Sigma Ltd. Nitro blue tetrazolium (NBT), riboflavin, L-methionine, thiobarbituric acid and ethylenediaminetetraacetic acid (EDTA) were purchased from Hi-Media Ltd.; ferric chloride anhydrous ($FeCl_3$), ascorbic acid, trichloroacetic acid and potassium persulfate were purchased from Merck Ltd. All reagents were of analytical grade.

3. Preparation of different extracts of *Pueraria tuberosa*

Tubers of *P. tuberosa* were collected from the Ayurvedic Garden, Department of Dravya Guna, Institute of Medical Science, Banaras Hindu University and their authenticity was compared with a reference sample preserved in the Department. The coarse powdered tuber was successively extracted with hexane and methanol in a continuous Soxhlet extractor.

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4. ABTS assay

ABTS radical-scavenging activity of *P. tuberosa* extract was determined, according to Re et al. (1999). ABTS radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in the dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. A standard was made up, containing 950 μl of ABTS solution and 50 μl of vitamin C, which was compared with 50 ml of extract in 950 μl of ABTS solution. The extracts and standard were vortexed for 10 s; after 6 min absorbance was recorded at 734 nm, using an ELICO (SL-150) UV–vis spectrophotometer. Distilled water was used as a blank.

5. Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay (Ohkowa, Ohisi, & Yagi, 1979) was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid-rich media (Ruberto, Baratta, Deans, & Dorman, 2000). Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA), yielding a pinkish red chromogen with an absorbance maximum at 532 nm (Janero, 1990). Egg homogenate (0.5 ml, 10% in distilled water, v/v) and 0.1 ml of extract were mixed in a test tube and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. For the blank 0.1 ml of distilled water was used in place of the extract.

6. Superoxide radical-scavenging property

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp & Fridovich, 1971). In brief, each 3 ml reaction mixture contained 0.01 M phosphate buffer (pH 7.8) (PBS), 130 mM methionine, 60 μM riboflavin, 0.5 mM EDTA, NBT (0.75 mM) and 0.5 ml of test sample solution. It was kept in front of a fluorescent light and absorbance was taken after 6 min at 560 nm. Identical tubes containing reaction mixture were kept in the dark and served as controls. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample. The blank was 0.01 M PBS.

7. Hydroxyl radical-scavenging assay

7.1. Non-site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation

Deoxyribose was used for determining the scavenging effect of *P. tuberosa* extracts on hydroxyl radicals, as described by Halliwell, Gutteridge, and Aruoma (1987). The reaction mixtures contained ascorbic acid (200 μM), $FeCl_3$ (200 μM), EDTA (200 μM), H_2O_2 (20 mM), deoxyribose (5 mM) with different concentrations of the test extracts in 1 ml of PBS (20 mM, pH 7.4). They were incubated at 37 °C for 1 h and then 1 ml of 2.8% TCA (w/v in water) and 1 ml of 1% TBA (w/v) were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was measured at 532 nm. Thiourea was the positive control and PBS was used as the blank.

7.2. Site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation

The ability of the extracts to inhibit site-specific hydroxyl radical-mediated peroxidation was also carried out, to understand the role of *P. tuberosa* as a metal chelator. The method was the same as described above, except that buffer was replaced by EDTA.

8. Measurement of total phenolics (TP)

TP concentration was measured by Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, 5 ml of distilled water, 0.5–1.0 ml of sample, and 1.0 ml of Folin–Ciocalteu reagent were added to a 25 ml flask. The contents were mixed and allowed to stand for 5–8 min at room temperature. Next, 10 ml of 7% sodium carbonate solution were added, followed by distilled water. Solutions were mixed and allowed to stand at room temperature for 15 min, and then absorbance was recorded at 750 nm. TP content was standardised against gallic acid and expressed as milligrams per litre of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5–5.0 mg/l GAE ($R^2 = 0.999$), giving an absorbance range of 0.050–0.555 absorbance units.

9. Statistics

All data are expressed as means ± SD. Pearson's correlation analysis (SPSS 7.5 for Windows, SPSS Inc.) was used to test for the significance of the relationship between the concentration and percentage inhibition.

10. Results

EC_{50} values (concentration of sample required to produce 50% response) were determined by the graph, prepared from the different concentrations of the extracts, versus percentage inhibition for the following parameters:

Table 1
Antioxidant activity of two fractions of tuber of *P. tuberosa* observed with ABTS radical

Concentration (μg/ml)	Percentage inhibition (mean ± SD)	
	Hexane extract	Methanol extract
50	28.1 ± 1.24	9.86 ± 0.7
100	57.6 ± 0.6	15.5 ± 1.3
200	71.2 ± 0.36	24.8 ± 0.92
300	84.6 ± 1.20	44.6 ± 1.68
500	98.4 ± 1.05	72.5 ± 1.08
700	98.2 ± 1.38	97.3 ± 1.5
1000	98.7 ± 1.55	98.1 ± 0.98
EC_{50}	120	330

EC_{50} (μg/ml) of vitamin C 45.20, $n = 3$. All the observations in different groups showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis).

(1) ABTS assay
There was greater inhibition by the hexane extract than the methanol extract, as the EC_{50} value of the hexane fraction was 98 μg/ml, as compared to the methanol fraction, whose EC_{50} value was 320 μmg/ml (Table 1).
(2) Lipid peroxidation assay
The two fractions of *P. tuberosa* inhibited lipid peroxidation, induced by ferrous sulfate in egg-yolk homogenates in a concentration dependent manner. Interestingly, there was no significant difference in the EC_{50} values of both fractions (Table 2).
(3) Superoxide scavenging assay
Both the fractions exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner with similar EC_{50} values (Table 3).
(4) Non-site-specific hydroxyl radical-scavenging assay
The methanolic fraction was found to be the more potent hydroxyl radical scavenger with an EC_{50} value of 310 μg/ml compared to the hexane fraction, whose EC_{50} value was 480 μg/ml.
(5) Site-specific hydroxyl radical-scavenging assay
The methanolic fraction was found to be the more potent hydroxyl radical scavenger, with an EC_{50} value of 89 μg/ml, compared to the hexane fraction, whose EC_{50} value was 120 μg/ml (Table 4). It was found to be more potent in this situation, which shows its role as metal chelator also. These results were compared with those for butylated hydroxytoluene (BHT) and quercetin (Table 5).
(6) Measurement of total phenolic (TP) content
The methanolic extract of *P. tuberosa* had a higher phenolic content than the hexane fraction. Its content was also higher than the methanolic extract of *Vitex negundo* (Tiwari & Tripathi, 2007) (Table 6).

Table 2
Inhibition of lipid peroxidation induced by $FeSO_4$ using egg-yolk homogenates as lipid-rich media by two fractions of tuber of *P. tuberosa*

Concentration (μg/ml)	Percentage inhibition (mean ± SD)	
	Hexane extract	Methanol extract
500	28.7 ± 0.806	25.2 ± 1.6
700	47.7 ± 1.45	40.8 ± 0.92
900	58.90 ± 1.33	67.8 ± 1.01
1000	68.9 ± 1.20	75.7 ± 0.81
1200	84.0 ± 1.12	82.9 ± 1.03
1400	96.7 ± 0.92	98.3 ± 0.72
2000	97.0 ± 0.81	98.1 ± 1.02
EC_{50}	720	780

EC_{50} of BHT 0.74 μg/ml, EC_{50} of quercetin 0.20 μg/ml, $n = 3$. All the observations in different groups showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis).

Table 3
Superoxide anion scavenging activity of two fractions of tuber of *P. tuberosa* observed with a riboflavin-light-NBT system

Concentration (μg/ml)	Percentage inhibition (mean ± SD)	
	Hexane extract	Methanol extract
100	$16.4 \pm 1.62^{*}$	$27.4 \pm 1.05^{**}$
200	$45.2 \pm 1.35^{*}$	$43.7 \pm 0.79^{**}$
300	$65.7 \pm 1.18^{*}$	$67.7 \pm 1.04^{**}$
400	$85.4 \pm 0.86^{*}$	$83.9 \pm 0.77^{**}$
500	$96.7 \pm 1.08^{*}$	$97.6 \pm 1.09^{**}$
700	$97.2 \pm 1.12^{*}$	$98.2 \pm 0.92^{**}$
EC_{50}	220	240

EC_{50} of $CuSO_4$ 41.6 μg/ml, EC_{50} of BHT 0.16 μg/ml, and EC_{50} of quercetin was 0.19 μg/ml; $n = 3$. All the observations in different groups showed significant ($P^{*} < 0.05$ and $P^{**} < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis).

Table 4
Hydroxyl radical-scavenging activity of two fractions of tuber of *P. tuberosa* in the deoxyribose assay in the presence and absence of EDTA

Concentration (μg/ml)	Percentage inhibition (mean ± SD)			
	Hexane extract		Methanol extract	
	Non-site-specific	Site-specific	Non-site-specific	Site-specific
100	$5.86 \pm 1.50^{*}$	$27.2 \pm 0.63^{*}$	$32.9 \pm 0.45^{**}$	$47.0 \pm 0.52^{**}$
300	$21.8 \pm 0.83^{*}$	$68.1 \pm 0.45^{*}$	$45.3 \pm 0.50^{**}$	$65.5 \pm 1.04^{**}$
500	$57.6 \pm 1.02^{*}$	$76.1 \pm 1.01^{*}$	$62.5 \pm 1.02^{**}$	$77.8 \pm 1.02^{**}$
700	$89.0 \pm 1.4^{*}$	$98.92 \pm 0.78^{*}$	$81.3 \pm 1.02^{**}$	$98.7 \pm 0.95^{**}$
1000	$92.1 \pm 1.1^{*}$	$99.32 \pm 0.87^{*}$	$94.1 \pm 0.64^{**}$	$99.5 \pm 1.03^{**}$
EC_{50}	480	210	310	120

EC_{50} of BHT: non-site-specific 0.56 μg/ml and site-specific, 0.18 μg/ml. EC_{50} of quercetin: non-site-specific 0.20 μg/ml and site-specific 0.17 μg/ml; $n = 3$. All observations in different groups showed significant ($P^{*} < 0.05$ and $P^{**} < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis).

11. Discussion

Both fractions of *P. tuberosa* exhibited a potent scavenging activity for ABTS radical cation in a concentration dependent manner, showing the direct role of *P. tuberosa* in trapping free radicals. It is known that lipid peroxidation is the net result of any free radical attack on membrane and

Table 5
Comparison of EC_{50} values of BHT and quercetin on different antioxidant parameters

Standard compound	EC_{50}			
	Lipid peroxidation	Superoxide scavenging	Hydroxyl radicals	
			Non-site-specific	Site-specific
BHT	3.4 μM (0.74 μg/ml)	0.74 μM (0.16 μg/ml)	2.56 μM (0.56 μg/ml)	0.82 μM (0.18 μg/ml)
Quercetin	0.6 μM (0.20 μg/ml)	0.59 μM (0.19 μg/ml)	0.6 μM (0.20 μg/ml)	0.51 μM (0.14 μg/ml)

Table 6
Total phenolic content in different fraction of *P. tuberosa* and methanolic fraction of *V. negundo*

Extract	Total phenolic content (mg of GAE/g)
P. tuberosa (methanolic)	2.34 ± 0.097
P. tuberosa (hexane)	1.21 ± 0.102
V. negundo (methanolic)	1.57 ± 0.094

other lipid constituent present in the system. It may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Since we have used egg-yolk as a substrate it could be suggested that *P. tuberosa* is active against non-enzymatic oxidation. This inhibition of lipid peroxidation may be either due to chelation of Fe or by free radical trapping.

Removal of superoxides in a concentration dependent manner by *P. tuberosa* fractions may be attributed to the direct action of its constituent phytomolecules on these radicals. Similarly, hydroxyl radical is the most reactive radical, which abstracts hydrogen atoms from biological molecules, including thiols, leading to the formation of sulfur radicals capable to combine with oxygen to generate oxysulfur radicals, a number of which damage biological molecules (Halliwell, 1991).

Our results show that the methanolic extract of *P. tuberosa* showed better trapping potential for hydroxyl radicals, compared to the hexane fraction and this may be attributed to the higher phenolic content of this fraction. The hexane fraction ABTS may be a better scavenger, due to the presence of non-phenolic antioxidant phytomolecules in that fraction.

Both *P. tuberosa* extracts exhibited strong ability to bind with iron, and a moderate potential for direct scavenging action of the OH radical. The extracts make a strong complex with iron, which is less reactive for generating OH radical, as compared to Fe–deoxyribose complex.

The lower activity of the *P. tuberosa* fractions compared to quercetin and BHT could be associated with lesser polyphenol content and other antioxidant compounds in the *P. tuberosa* fractions. After viewing the structure of the reported compounds of *P. tuberosa* extract, it appears that these compounds have 2–3 free hydroxyl groups, whereas, in quercetin there are five free phenolic groups. The other possibility for the lower activity of the extracts could be associated with the glycosidic nature of its reported compounds, because glycosidic substitution reduces the number of free hydroxyl groups in the polyphenols, which is mainly responsible for its antioxidant potential.

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References

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*, 276–287.

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). *Glossary of Indian Medicinal Plants*. New Delhi: CISR.

Halliwell, B. (1991). Reactive oxygen species in living systems: Source, biochemistry and role in human disease. *Analytical Journal of Medicine, 91*(Suppl. 3C), 14S–22S.

Halliwell, B., Gutteridge, 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*, 215–219.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine, 9*, 515–540.

Ohkowa, H., Ohisi, N., & Yagi, K. (1979). Assay for lipid peroxides in animals tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.

Pandey, G. S., & Chunekar, K. C. (Eds.). (1998). Bhav Prakash Nighantu (pp. 388–389). Varanasi: Chaukambha Vidya Bhavan.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant acitivity applying an improved ABTS radical cation decolorizing assay. *Free Radicals in Biology and Medicine, 26*, 1231–1237.

Ruberto, G., Baratta, M. T., Deans, S. G., & Dorman, H. J. D. (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Medica, 66*, 687–693.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). *Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in enzymology, oxidants and antioxidants, part A* (Vol. 299). San Diego, CA: Academic Press.

Tiwari, O. P., & Tripathi, Y. B. (2007). Antioxidant properties of different fractions of *Vitex negundo* Linn. *Food Chemistry, 100*, 1170–1176.