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Full Length Research Paper

Assessment of antioxidative activities of extracts from selected *Plantago* species

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Majority of the worlds population is still relying on natural products, infact synthetic drugs have revolutionized the modern pharmaceuticlas. *Plantago* have wide range of bioactive compounds. This study involved antioxidative activities of extracts from three *Plantago* species (*Plantago ovata, Plantago lanceolata* and *Plantago maxicana)* by ascorbate assay and catalase assay. All extracts showed antioxidant activities in both assays except for *P. maxicana* in catalase assay. *P. ovata* was observed to be the most active in both assays indicating highest antioxidant activity among the selected *Plantago* species. The current study would be beneficial to explore further about the valuable compounds.

Key words: Plantago, antioxidants, ascorbate assay, catalase assay.

INTRODUCTION

The occurrence of *Plantago* is worldwide and comprising 131 species. It is perennial herb; leaves are basal, flowers small, white dense florescence, seeds are dull black and endopsermous (Watson and Dallwitz, 1992). It is being considered as a rich in secondary metabolites including; hidpidulin, neochlorognic acid, scutellarein, catalpol, aucabin, ursolic acid. The Plantago species is being used as a medicine since long time in herbal formulations and found effective to cutaneous leishmaniasis, cure chronic constipation, digestive disorders, diarrhea, piles and alleviated problems of kidney, bladder and hemorrhoids. The research have proven that *Plantago* has important properties like anti-inflammatory, anti-colocon cancer, analgesic, antibacterial, antioxidant, immunomodulatory, proliferative, antimutagenic, wound healing (Juarranz et al., 2002; http://www.altnature.com/gallery/plantain.htm). It has also roles in the regulation of blood cholesterol without major changes in triglycerides and reduces blood glucose (ESCOP, 1997).

Free radicals in living systems are the causative agent of cancer, rheumatism, stroke, aging, diabetes mellitus and coronary heart disease (Wong et al., 1987; Halliwell, 1997; Droge, 2002). It has been reported that reactive oxygen species (ROS) like $1O_2$ (singlet oxygen), (superoxide anion) which are involved in internal injuries of cells and they may also recruit the peroxidation of polyunsaturated fatty acids in bio membranes (Compori, 1985; Halliwell, 1997). However, aerobic living systems are much safer from oxygen toxicity with the help of naturally existing antioxidant defense system (Cotgreave et al., 1988; Ames et al., 1993).

Antioxidative activities of methanol extracts from five *Plantago* species (*Plantago afra, Plantago coronopus, Plantago lagopus, Plantago lanceolata, and Plantago serraria*) were characterized by the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging test and the inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation on bovine brain liposomes. All extracts showed antioxidant activity in both methods. Whereas *P. serraria* exhibited the strongest activity as a DPPH scavenger, *P. lanceolata* and *P. serraria* were found to be the most active in the lipid peroxidation inhibition assay. It was observed that *P. serraria* is presented as a possible new source of natural antioxidants (Galvez et al., 2005). Keeping in view the importance of *Plantago* species, current study was conducted to evaluate the antioxidant activities of three *Plantago* species.

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MATERIALS AND METHODS

Plant material

Three *Plantago* species namely *P. ovata, P. lanceolata* and *P. maxicana* were used as plant material for antioxidative analysis. The plant material was collected from National Agriculture Research Centre, Islamabad, Pakistan.

Preparation of stock solutions

Phosphate buffer pH 7

For the preparation of phosphate buffer (pH 7) monobasic and dibasic solutions were prepared. Monobasic solution was prepared by using 15.6 g of sodium dihydrogen phosphate dissolved in 500 ml of distilled water. Dibasic solution was prepared by using 26.83 gm of disodium hydrogen phosphate dissolved in 500 ml of distilled water. Then 58.5 ml of monobasic solution and 91.5 ml of dibasic solution were mixed to prepare phosphate buffer of pH 7.

3% H_2O_2

500 ml of 3% H_2O_2 solution was prepared by using 150 ml of 30% H_2O_2 dissolved in 350 ml of distilled water.

50 µM Ascorbic acid solution

This solution was prepared by weighing out 0.00088 g of ascorbic acid powder and then was dissolved in 100 ml of distilled water.

Enzyme extract preparation

Enzyme extract was prepared by grinding 0.2 g of plant material with the help of chilled pestle and mortar by adding 4 ml of phosphate buffer pH 7 mixed with polyvinylpyrrolidone (PVP 1%). The crushed material was then transferred to eppendrof tubes and centrifuged for 10 to 15 min at 4000 rpm. Supernatant was poured into a new eppendorf.

Ascorbate assay

Antioxidant capacity was observed by following the protocol set by Asada and Takahashi (1987). For ascorbate assay 1 ml of phosphate buffer (pH 7) was used as blank reading for spectrophotometer. Then, 0.1 ml of enzyme extract was taken in a test tube and mixed with 2 ml of phosphate buffer (pH 7) and 0.2 ml of 50 µM ascorbic acid solution.

Then 0.2 ml of 3% H_2O_2 was added to the previous mixture and immediately after adding H_2O_2 optical density was measured at 290 nm as initial reading. Another reading of the same sample was taken after 180 s as final reading. These two readings were recorded and same procedure was repeated for three replicas of enzyme extract of each sample.

Catalase assay

Antioxidant activity was monitored using protocol set by Chandlee and Schanalios (1984). For catalase assay 1 ml of phosphate buffer (pH 7) was used as blank reading for spectrophotometer. 0.2 ml of enzyme extract was taken in a test tube and mixed with 0.5 ml of phosphate buffer (pH 7). Then 0.1 ml of 3% H_2O_2 was added to the previous mixture and immediately after adding H_2O_2 optical density was measured at 240 nm as initial reading. Another reading of the same sample was taken after 180 s as final reading. These two readings were recorded and same procedure was repeated for three replicas of enzyme extract for each sample.

RESULTS AND DISCUSSION

As the values in Table 1 clearly indicate that inhibition of ascorbic acid peroxidation of plant extracts decreased in the following order *P. ovata* < *P. lanceolata* < *P. maxicana.* The results revealed that the inhibition of peroxidation was progressively greater in *P. ovata* as compared to other two species. The data mentioned in Table 2 is an indicative of the inhibition of peroxidation by hydrogen peroxide of plant extracts, which is decreasing in the following order *P. ovata* < *P. lanceolata* < *P. maxicana*. The results revealed that the inhibition of peroxidation was positive in two species and negative in one; in *P. maxicana* that is, showing no activity as compared to other two species. In *P. ovata* antioxidant activity was moderate which is quiet close to the result of *P. lanceolata*. Column chart have been used to provide the visual demonstration of over all antioxidant activity revealed by each species using ascorbate assay and catalase assay (Figure 1). Each column in the chart indicates the antioxidant strength exhibited by each species against a particular enzyme. Mean values were used to plot the column chart.

As mentioned in Figure 1 first two columns represent antioxidant activity of *P. ovata*, over all high antioxidant activity was exhibited that is, for ascorbic acid and for catalase. Third and forth columns represent antioxidant activity of *P. maxicana*. In case of ascorbic acid, it showed moderate antioxidant activity and in case of catalase it showed negative activity. Fifth and sixth columns represent antioxidant activity of *P. lanceolata*, comparatively it revealed moderate antioxidant activity among three species. In case of ascorbic acid, high antioxidant activity was observed while in case of catalase it showed moderate activity.

DISCUSSION

According to Fraga et al. (1991), ascorbate is the most studied antioxidant vitamin for its role in reducing the risk of degenerative diseases. Recent studies have shown that plants total phenolics and anthocyanins also contribute to the antioxidant capacity along with ascorbate (Kalt et al., 1999; Deighton et al., 2000; Connor et al., 2002; Kang et al., 2002). There are earlier reports regarding the estimation of antioxidant activities in different plant. In mango, it was observed that the reduction in antioxidant capacity after a certain period of storage was found to be mainly due to its strong relationship with ascorbic acid rather than total phenols (Shiwashankara et al., 2003). In another report it was

Table 1. Readings for evaluation of antioxidant activity of plant extracts against ascorbic acid peroxidation.

Plantago species	Initial reading	Final reading	Initial – final / 3	Mean
	0.10	- 3.26	1.12	
Plantago ovata	0.25	- 5.26	1.83	2.03
	0.15	- 9.29	3.14	
	1.64	- 0.11	0.58	
Plantago maxicana	1.94	- 0.45	0.79	0.73
	2.01	- 0.52	0.84	
	0.26	- 3.62	1.29	
Plantago lanceolata	0.44	- 3.80	1.41	1.47
	0.91	- 4.27	1.72	

Table 2. Readings for evaluation of antioxidant activity of plant extracts against hydrogen peroxide.

Plantago species	Initial reading	Final reading	Initial – final / 3	Mean
	0.55	- 0.98	0.51	
Plantago ovata	0.20	- 0.63	0.27	0.39
	0.39	- 0.82	0.40	
	0.33	2.00	- 0.55	
Plantago maxicana	0.66	7.01	- 2.11	- 1.67
	0.41	7.46	- 2.35	
	0.31	- 0.95	0.42	
Plantago lanceolata	0.11	- 0.79	0.30	0.36
	0.39	- 0.70	0.36	

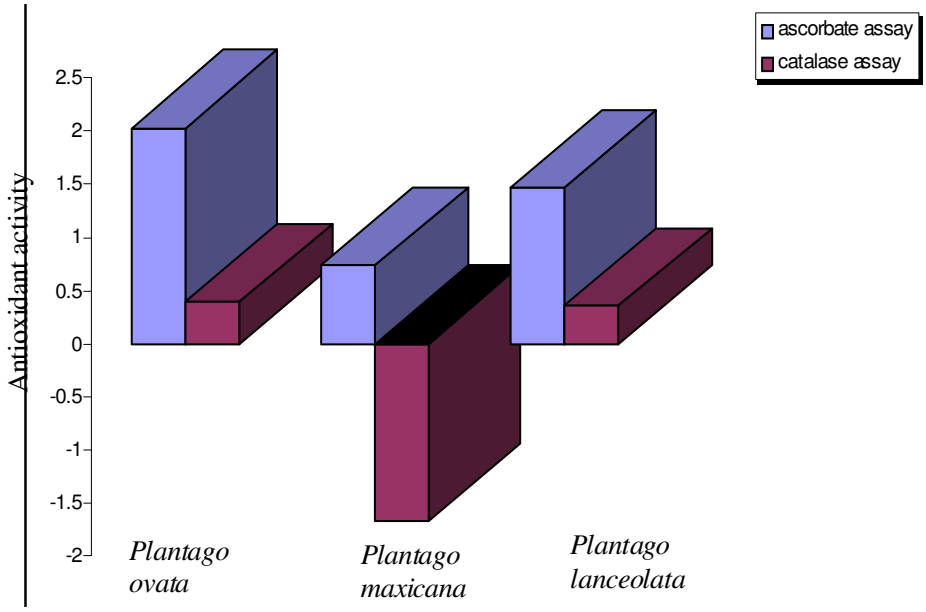

Figure 1. Column chart representing the mean values obtained against both ascorbic acid and catalase enzymes.

observed that total ascorbic acid content in *C. asiatica*, *P. sarmentosum* and *K. galanga* has higher antioxidant activity than total ascorbic acid (Chanwitheesuk et al., 2005). The authors also observed that vegetable derived from *P. sarmentosum* and *K. galanga* family showed a higher correlation of total ascorbic acid content with total antioxidant content. Based on the correlation between ascorbate content and antioxidant capacity, present study was carried out to find ascorbate content and to correlate it with antioxidant capacity of three *Plantago* species. It was found by study that ascorbate content is highest based on absorption rate in *P. ovata,* indicating that its antioxidant capacity is higher. Moreover, it was revealed that *P. maxicana* exhibited moderate and *P. lanceolata* showed high antioxidant capacity based on ascorbate content, which is in accordance with the previously reported study of Galvez et al. (2005) who worked on antioxidative activities of five *Plantago* species by DPPH scavenging test and ascorbate induced lipid peroxidation on brain liposomes; *P. lanceolata* in this report study was found to be active in lipid peroxidation inhibition assay.

Further, the present study also involves the analyses of antioxidant enzyme that is, catalase content of *Plantago* species for evaluating antioxidant capacity. According to the work of Qujeq and Rezvani (2007) an imbalance in antioxidant enzymes has been related to specific complications such as diabetic problems. Catalase catalyzes the reduction of hydroperoxides, thereby protecting mammalian cells against oxidative damage. In addition, catalase is active in neutralizing reactive oxygen species (ROS) and removes cellular superoxide and peroxides before they react with metal catalysts to form more reactive species (Qujeq and Rezvani, 2007). It was revealed by Sun et al. (1984) that catalase is easily inactivated by lipid peroxides or ROS. Based on the observed data, it can be depicted that catalase content of studied *Plantago* species is moderate. It was observed that *P. ovata* has the highest catalase content, immediately followed by *P. lanceolata* which is not far behind in observed values. However, on the basis of catalase content, no antioxidant activity was monitored in case of *P. maxicana*.

Conclusion

It was concluded from the present study that *Plantago* species has shown good antioxidant activity. Moreover, based on ascorbate and catalase assay it can be resolved that all *Plantago* species do not exhibit the antioxidant activity against all enzymes. Selected *Plantago* species tested for oxidative analysis revealed that *P. ovata* and *P. lanceolata* has antioxidant activity using both assays while *P. maxicana* showed negative result against catalase enzyme. *P. ovata* explicitly exhibited high antioxidant capacity using both assays among the selected Plantago species. However, there is a need to study bioactive compounds in *Plantago* by modern techniques to exploit natural products in a more effective manner.

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