Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

A PRELIMINARY STUDY ON THE *IN VITRO* ANTIOXIDANT ACTIVITY OF SEEDS OF *Aesculus indica and* BARKS OF *Populus euphratica*

DILIPKUMAR PAL[1]*, HARPREET SINGH[2] AND MANOJ KUMAR[2]

[1]Department of Pharmaceutical Sciences, Guru Ghasidash Vishwavidyalya (A Central University), Koni, Bilashpur 495009, C.G., India, [2]College of Pharmacy, Institute of Foreign Trade and Management (IFTM), Lodhipur Rajput, Delhi Road, Moradabad 244001, U.P., India. Email: drdilip2003@yahoo.co.in

Received: 16 July 2012, Revised and Accepted: 02 Sep 2012

ABSTRACT

The *in vitro* antioxidant activity of seeds of *Aesculus indica and* barks of *Populus euphratica* had been investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520 nm. It was found that ethanol & chloroform extract of seeds of *A. indica and* ethanol & aqueous extract of barks of *P. euphratica* had better antioxidant activity than other extracts of the respective plant. The antioxidant activities of the extracts were close and comparable to that of standard antioxidant compounds used.

Keywords: Antioxidant activity, *Aesculus indica*, *Populus euphratica*, Non-enzymatic haemoglobin glycosylation.

INTRODUCTION

Herbal medicine has become an increasingly common form of alternative therapy. Twelve percent of adults in the United States had used an herbal medicine in the previous 12 months. But the use of some herbal medicines and its control are not based on proper scientific basis. Recently, a great deal of interest has been directed towards the searching of different types of antioxidants as they have preventive role in the field of cancer and other diseases[1-2]. Keeping this in view, the present communication deals with the study of *in vitro* antioxidant activity of seeds of *Aesculus indica* L. and barks of *Populus euphratica*.

Aesculus indica (Indian Horse chestnut, family: Hippocastanaceae) is a large, rounded tree found in the Himalayas and Northern India. Seeds of *A. indica* are used in traditional Indian medicine for the treatment of skin diseases, rheumatism and headaches. It is used as an astringent, acrid and narcotic. Its large leaves and flowers make it suitable for use as large sized bonsai. Its leaves are used as cattle fodder in parts of Northern India. The fruits are given to horses suffering from colic. The nuts are used in piles and obstinate constipation. An extract of leaves has been found to be useful in whooping-cough[3-5].

Populus euphratica (commonly known as Indian poplar, Bhan in Hindi & Punjabi, family: Salicaceae) is a large, tall tree found abundantly in subtropical regions, Western Himalayas including Punjab. Barks of *P. euphratica* are used as vermifuge. Bark and leaves of it also have antichloristic, analgesic, antibacterial and spasmolytic effects[6-9].

Evaluation of the antioxidant activity of any drug sample or herbal extract can be carried out either by *in vitro* or *in vivo* models. Here, the evaluation was carried out by *in vitro* non-enzymatic glycosylation of haemoglobin method. Since non-enzymatic glycosylation of haemoglobin is an oxidation reaction, an antioxidant is expected to inhibit the reaction. The degree of haemoglycosylation *in vitro* in the presence of different concentration of extracts can be measured colorimetrically.

MATERIAL AND METHODS

Chemicals

Haemoglobin was purchased from Nice Chemicals Pvt. Ltd., Cochin. Glucose, phosphate buffer and D-α-tocopherol were procured from Merck, Mumbai. Ascorbic acid and gentamycin were obtained from Biokem International Pvt. Ltd., Bangalore and Nicholas Piramol India Ltd., Pithampur respectively. All other reagents and solvents used were of analytical grade.

Preparation of extracts

The fresh seeds of *A. indica* were collected from hill area of Ranikhet Jungle (Uttarakhand) in the month of September from Moradabad, U.P., India. The barks of P. euphratica were collected from the fields of Sambhal region (Moradabad district) in the month of September-October. The plant was authenticated by Dr. D.V. Amla, Scientist-G, National Botanical Research Institute, CSIR (Govt. of India), Lucknow-226001**.** The voucher specimens have been preserved in LWG Harbarium of NBRI, Lucknow. Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60^0 C), chloroform, ethyl acetate, ethanol and distilled water using a soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and water extracts of *A. indica* and *P. euphratica* were 2.4, 6.0, 7.4, 3.3, 0.5 and 2.6, 3.6, 1.8, 2.4, 2.6 % w/w respectively. The extracts were subjected to antioxidant studies.

Phytochemical Screening

The phytochemical examinations of seeds of *A. indica and* barks of *P. euphratica* were performed by the standard methods[10-12].

Antioxidant studies

Non-enzymatic haemoglycosylation method

The antioxidant activities of different extracts were investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically. Haemoglobin, 60 mg/100 mL in 0.01 M phosphate buffer (pH 7.4) was incubated in presence of 2 g/100 mL concentration of glucose for 72 h in order to find out the best condition for haemoglobin glycosylation. The assay was performed by adding 1 mL of glucose solution, 1 mL of haemoglobin solution and 1 mL of gentamycin (20 mg/ 100 mL) in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of hemoglobin in the presence of different concentration of extracts and their absence were measured colorimetrically at 520 nm[13-18].

RESULTS AND DISCUSSION

Phytochemical screening indicated the presence of flavonoids, tannins & saponin in ethanol and glycosides in chloroform extract of *A. indica*. Again, alkaloids, tannins, flavonoids in ethanol extract and alkaloids, tannins in aqueous extract of *P. euphratica* were also positive on qualitative tests.

Results of antioxidant activity of seeds of *A. indica and* barks of *P. euphratica* are summarized in Table 1. The results obtained indicated that ethanol and chloroform extract of seeds of *A. indica*

had better antioxidant activity than petroleum ether, ethyl acetate and aqueous extract estimated by haemoglobin glycosylation with a concentration of 1.0 mg/ml of each.

Table 1: Antioxidant activity of different extracts of *A. indica and P. euphratica*

Samples	Final concentration of the tested compound (mg/mL)	
	0.5	**1.0**
PEAI	32.7±0.16	62.5±0.35
CEAI	36.0±0.18	70.3±0.45
EAAI	28.9±0.27	57.1±0.40
EEAI	38.5±0.79	76.9±0.92
AEAI	22.9±0.35	45.5±0.53
PEPE	17.0±0.18	33.3±0.27
CEPE	31.1±0.42	62.5±0.78
EAPE	29.8±0.18	57.1±0.51
EEPE	47.1±0.50	92.7±0.97
AEPE	43.0±0.35	85.7±0.69
D-α- tocopherol	9.5±0.12	18.9±0.38
Ascorbic acid	11.8±0.24	6.0±0.13

Abbreviations: Percent inhibition of haemoglobin glycosylation was measured at two concentrations of PEAI, CEAI, EAAI, EEAI, AEAI (petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract of *A indica* respectively) and PEPE, CEPE, EAPE, EEPE, AEPE (petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract of *P. euphratica, r*espectively) . The activities were compared with those of D-α- tocopherol and ascorbic acid. Values are mean ± S.E.M. of three replicates.

It was also observed that ethanol and aqueous extract of barks of *P. euphratica* had better antioxidant activity than petroleum ether, ethyl acetate and chloroform extract estimated by the same process with same concentration. It was also found that the antioxidant activities of the extracts were concentration dependent. The activities were compared with D-α- tocopherol (vitamin E) and ascorbic acid (vitamin C) that were used as standard antioxidant compounds.

Preliminary phytochemical investigations indicated the presence of flavonoids, tannins in seeds of *A. indica* and alkaloids, tannins, flavonoids in barks of *P. euphratica*. These compounds may be responsible for providing better antioxidant activity in respective extracts[13,16]. The detailed chemical nature of the active principle (s) responsible for antioxidant activity and their mode of action are under investigation.

REFERENCES

1. Pal DK, Saha S, Singh S. Importance of pyrazole moiety in the field of cancer. Int J Pharm Pharm Sci 2012; 4(2), 98-104.
2. Pal DK. Determination of brain biogenic amines in *Cynodon dactylon* Pers. and *Cyperus rotundus* L treated mice. Int J Pharm Pharm Sci 2009; 1(1), 190-197.
3. Evans WC. Trease and Evan's Pharmacognosy, 3rd Edition, London: ELBS; 1994. p. 161-163.
4. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 39th Edition. Pune: Nirali Prakashan; 2003, p. 5, 112.
5. Rana TS, Datt B. An Ethnobotanical Observation among Jaunsaries of Jaunsar- Bawar, Dehradun (U.P.) India, Int J Oharmacog 1997; 35: 371-374.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol III, Rajput Road, Dehradun: International Book Distributors, Book Sellers and Publishers; 2005, p. 2369.
7. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Pune: Nirali Prakashan; 2008, p. 149-161.
8. Kalia AN. Text Book of Industrial Pharmacognosy. 1st Edition, New Delhi: CBS Publishers and Distributors; 2005, p. 1-2.
9. The Wealth of India: A Dictionary in Indian Raw Material Industrial Products, Vol 3, New Delhi, India: Publication and Information Directorate, CSIR; 1997, p. 214.
10. Fluckiger R, Winterhalter KH. Biochemical and Chemical Aspects of Haemoglobin Abnormalities. New York : Academic Press; 1978, p.208.
11. Dinda B, Ghosh B, Arima S, Sato N, Harigaya Y. Phytochemical investigation of *Gomphrena globosa* aerial parts. Indian J Chem, 2004; 42B: 2223.
12. Anthony AE., Anthony FF, Peter AL, Darek JM. Triterpenoid saponin from fruit of *Lagenaria reviflora*. Phytochem 1990; 29: 3281-3285.
13. Pal DK, Dutta S. Evaluation of antioxidant activity of the roots of *Cyperus rotundus* L. Indian J Pharm Sci 2006; 68: 256-258.
14. Pal DK., Kumar M, Chakraborty P, Kumar S. Evaluation of antioxidant activity of aerial parts of *Cynodon dactylon*. Asian J Chem 2008; 20: 2479-2481.
15. Sannigrahi S, Mazumder UK, Pal DK, Parida S. In vitro antioxidant activity of methanol extract of *Clerodendrum infortunatum*. OPEM 2009; 9: 128-134.
16. Yadav SB, Tripathy V, Singh RK, Pandey HP. Antioxidant activity of *Cuscuta reflexa* stems. Indian J Pharm Sci 2000; 6: 477-478.
17. Pal DK, Nimse SB. Screening of antioxidant activity of *Hydrilla verticillata* plant. Asian J Chem 2006; 18: 3004-08.
18. Pal DK, Kumar S, Chakraborty P, Kumar M. A study on the antioxidant activity of *Semecarpus anacardium* L. f. nuts. J Nat Rem 2008; 8: 160-163.