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Research Article

REDUCING POWER OF THE SOLVENT EXTRACTS OF *EICHHORNIA CRASSIPES* (MART.) SOLMS

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ABSTRACT

Reducing power assay of solvent extracts of *Eichhornia crassipes* (Mart.) Solms at different concentrations and time delay was evaluated. Reducing power was linearly proportional to the concentration and time and was found to increase with increase in concentration and time. The extracts were compared to standard antioxidant L-ascorbic acid. All extracts showed greater reducing power than that of the standard. The results suggest the potential of development of useful natural antioxidants.

Keywords: *Eichhornia crassipes*, Antioxidant

INTRODUCTION

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid eroxides. These free radicals may either be produced by physiological or biochemical processes or by pollution and other endogenous sources. All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage[1].

Antioxidants prevent the human system by neutralizing the free radicals interactively and synergistically. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity[2].

Eichhornia crassipes (Mart.) Solms is a free-floating perennial aquatic herb. Phytochemical studies carried out revealed the presence of flavanoids and other metabolites in the plant extract[3]. 2,5dimethoxy-4-phenylbenzoindenone isolated from water hyacinth was found to possess antimicotic activity[4]. 4,9-dimethoxy-7-phenyl-2,3dihydrophenalen-1-olo-O methyl ether, 4,9-dimethoxy-7-(4'-methoxy-phenyl)-2,3-dihidro-phenalen-l-olo-O methyl ether, 4,5-dimethoxy-9-phenyl-2,3-dihydro-phenalen-1-ol-O methyl ether which were isolated from water hyacinth was found to show antialgal activity[5]. This present study, therefore aimed at the evaluation of the reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms at various concentrations and time delay.

MATERIALS AND METHODS

Plant collection

Six tonnes of fresh water hyacinth was collected from Singanallur boat house, Coimbatore, Tamilnadu. The root portion was cut off and the plant was washed thoroughly to free from debris. The leaves and shoot portion were shade dried for 20 days. The dried plant material was sliced, ground coarsely and stored for further use.

Preparation of extracts

Water hyacinth (11/2kg) was defatted twice with petroleum ether (20L) for 6 hours and then twice with ethanolic KOH (17L) for 6 hours. The extract was desolvetised under reduced pressure and the residue was extracted thrice with acetone under reflux for 1 hour. The acetone extracts were pooled and concentrated.

Water hyacinth (140kg) was extracted successively with ethyl acetate (800L), water (800L) twice for 6 hours. A small portion (11/2kg) of the plant residue was extracted with 1% hydrochloric acid (3L) for 6 hours.

Reducing power assay

Principle

The reducing power of petroleum ether(PE), ethyl acetate(EA), acetone (Ac)and hydrolysed extract (Hy) of *Eichhornia crassipes* was determined by the slight modification of the method of Oyaizu, (1986)[6]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + Ferrous chloride

Chemicals required

Potassium ferricyanide (1% w/v), phosphate buffer (0.2 M, pH 6.6), trichloro acetic acid (10%), ferric chloride (0.1%) and ascorbic acid (1%).

Phosphate buffer preparation

Dibasic sodium phosphate (37.50 ml of 0.2M) is mixed with 62.5 ml monobasic sodium phosphate and diluted to 100 ml with water.

Protocol for reducing power

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Reducing power was measured by varying the concentration of the extract and the contact time.

RESULTS AND DISCUSSION

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[7]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants[8]. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method

to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe^{3+} ion.

Standard curve of ascorbic acid is shown in Fig.1. The reducing power of petroleum ether, ethyl acetate, acetone and hydrolysed extract of *Eichhornia crassipes* (Mart.) Solms, as a function of their concentration is shown in Fig.2 and Fig.3. The reducing power of the extracts as a function of time is presented in Fig.4 and Fig.5. The reducing power of all the extracts increased with increase in concentration.

Fig.6 shows the reducing power of the standard ascorbic acid and the extracts at concentration 50µg/ml at 20min. At 50µg/ml concentration, the petroleum ether, ethyl acetate, acetone and acid extract showed absorbances of 0.12, 0.34, 0.6, and 0.44 respectively. Thus, all the extracts except PE exhibit a higher reducing ability than the standard. Also, the reducing ability was found to be time-dependent. With increasing time, the reducing ability of the extracts was found to increase except acetone extract. In this case, after 20 min, the reducing power was found to decrease after attaining a maximum value. This may be due to the decrease in the reducers which would have converted the Fe^{3+}/ferricyanide complex to the ferrous form within a short time. The reducers are no longer available for the conversion of the ferricyanide complex.

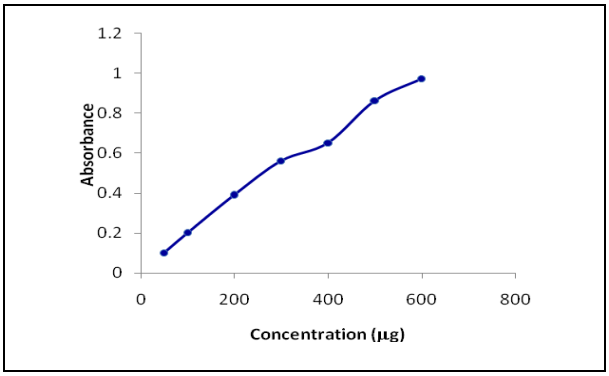

Fig. 1: Reducing ability of Ascorbic acid at various concentrations

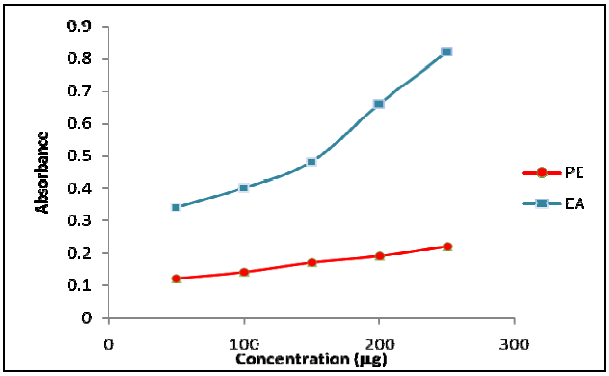

Fig. 2: Reducing ability of the petroleum ether and ethyl acetate extract as a function of concentration at constant time (20min)

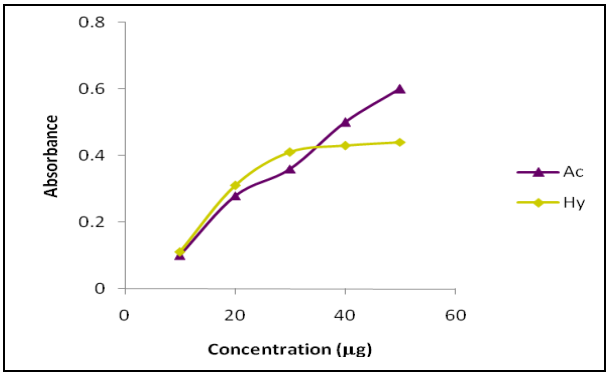

Fig. 3: Reducing ability of acetone and hydrolysed extract as a function of concentration at a constant time (20min)

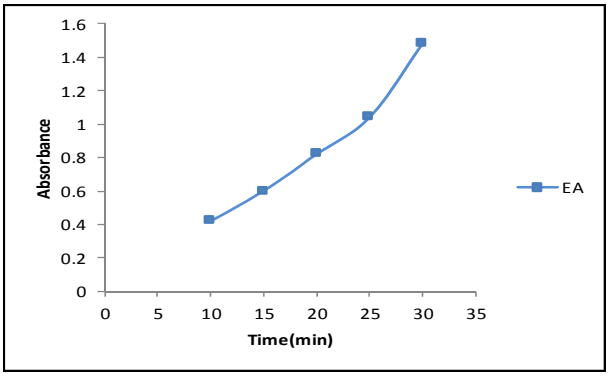

Fig. 4: Reducing ability of the petroleum ether and ethyl acetate extract as a function of time at concentration 250µg/ml

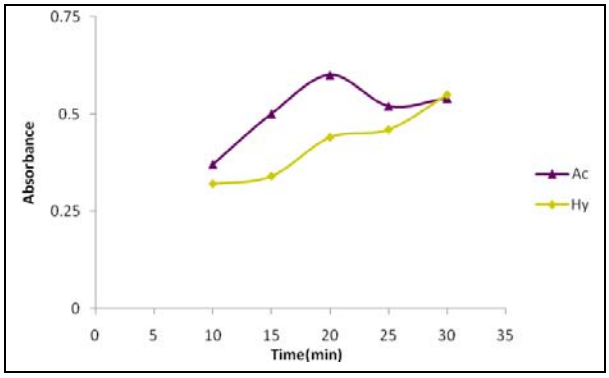

Fig. 6: Reducing power of the standard ascorbic acid and the extracts at concentration 50µg/ml at 20min

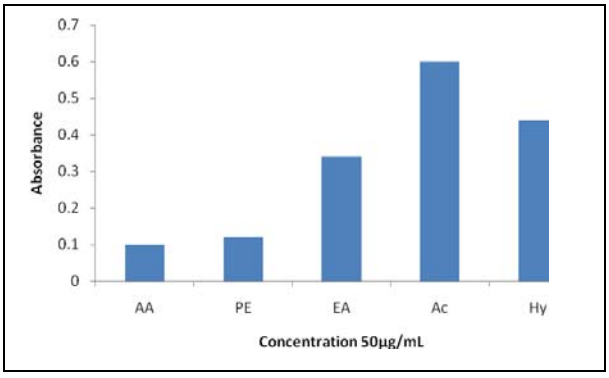

Fig. 5: Reducing ability of acetone and hydrolysed extract as a function of time at concentration 50µg/ml

CONCLUSION

Higher absorbance of the reaction mixture indicates higher reductive potential. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect. Also a correlation between the reducing power and antioxidant activity can be derived. In the present investigation, we have warranted the concentration dependent reducing ability of the extracts of *Eichhornia crassipes* (Mart.) Solms.

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