Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2012, 3 (2):271-277

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Study of antioxidant activity of ethanolic extract of fresh *Eichhornia crassipes* (Mart.)Solms

Thamaraiselvi, P. Lalitha* and P. Jayanthi

Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, Tamil Nadu, India

---

ABSTRACT

Waterhyacinth (Eichhornia crassipes) is a fast growing perennial aquatic macrophyte. It is well known to absorb and accumulate a large number of heavy metals and organic pollutants. The present study was designed to evaluate the antioxidant activity of ethanol extract of fresh Eichhornia crassipes by two different methods-reducing power assay and DPPH assay. Reducing power assay was evaluated at different concentrations and time delay. The reducing power of extract increases with increasing concentration of extract and 50µg/ml of extract gave 0.67 absorbance at 680 nm, comparable with the absorbance of ≈400µg/ml of standard (ascorbic acid). With variation in time, absorbance increased. Maximum absorbance (0.88) was noted with 10µg/ml of the extract in 30 min which was comparable to the absorbance of 500µg/ml of ascorbic acid. The free radical scavenging activity of 29µg of the ethanol extract was found to be comparable to the activity of 400µg of ascorbic acid. The result shows the potential of waterhyacinth as a source of useful natural antioxidants.

Key words: *Eichhornia crassipes*, antioxidant activity, ethanolic extract.

INTRODUCTION

Free radicals are produced in large amount during metabolic disease conditions like diabetes, hypertension, atherosclerosis, urolithiasis, ulcers etc. Oxidative free radicals are generated by metabolic reactions [1]. These free radicals attack DNA, protein molecules, enzymes and cells leading to change in genetic material and cell proliferation [2]. The use of compounds with antioxidant activity is expected to be useful for the treatment of these diseases [3]. Herbal medicines are frequently considered to be less toxic and more free from side effects than synthetic ones [4]. Several epidemiological, clinical and experimental data suggest that plant based antioxidants have beneficial effects on prevention on chronic diseases [5]. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programmes because these drugs are easily available at low cost and these are safe and people have faith in them [6].

Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity [7]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. The role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may cause many diseases including cancer [8].

Eichhornia crassipes (Mart.) Solms is a free-floating perennial aquatic herb. It possesses nutritionally important compounds like phenolics, flavonoids, glutathione and many other metabolites [9]. Phytochemical studies carried out revealed the presence of flavonoids and other metabolites in the plant extract [10]. These phytochemicals may have been responsible for antioxidant activity. Hence the present project was aimed to evaluate the antioxidant activity of ethanol extract of fresh *Eichhornia crassipes* by two different methods-reducing power assay and DPPH assay. Reducing power assay was evaluated at different concentrations and time delay.

MATERIALS AND METHODS

Eichhornia crassipes was collected from the lake near Ukkadam at Coimbatore. The plant sample was identified by Dr.J.Sudhakar, Botanical survey of India, Coimbatore. The root portion was cut off and the plant was washed thoroughly under running tap water to free from debris. The leaves and shoot portion of the fresh plant material was chopped into small pieces. The plant material was extracted with ethanol and desolvetised yielding ethanol extract.

Antioxidant activity

Reducing power assay

Ethanolic extract of fresh *Eichhornia crassipes* was subjected to reducing power activity by using the standard procedure [11].

Principle

The reducing power of ethanol extract of fresh *Eichhornia crassipes* was determined by slight modification of the method of Oyaizu [12]. 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 680nm.

Potassium ferricyanide + ferric chloride → potassium ferrocyanide + ferrous chloride

Chemicals required

- Potassium ferricyanide (1% w/v)
- Phosphate buffer (0.2M, pH 6.6)
- Trichloro acetic acid (10%)
- Ferric chloride (0.1%)
- Ascorbic acid (1%)

Phosphate buffer preparation

0.2M dibasic phosphate (35.61g in 1L) and 0.2M monobasic sodium phosphate (31.21g in 1L) was prepared. Dibasic sodium phosphate (18.75ml of 0.2M) was mixed with 31.25ml monobasic sodium phosphate and this was diluted to 100ml with water.

Procedure

The various concentrations of the extracts in ethanol were mixed with 2.5ml of phosphate buffer and 2.5ml of potassium ferricyanide. This mixture was kept in water bath at 50^{o}C for 20min. Aliquots of tricholoro acetic acid (2.5ml) were added to the mixture. The above solution (1ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 680 nm. A blank was prepared without adding extracts. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

DPPH assay (2, 2 – diphenyl – 1- picrylhydrazyl)

Ethanolic extract of fresh *Eichhornia crassipes* was subjected to DPPH assay by using the standard procedure [11].

Principle

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as

DPPH + (H - A) → DPPH - H + A

(Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as a consequence the absorbance's decrease from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Chemicals required

- Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol.
- Ascorbic acid 1%

Procedure

A solution of DPPH (0.1 mM) in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (1-16µg /ml). Thirty minutes later the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity.

RESULTS AND DISCUSSION

Reducing power assay

The reductive capabilities of the ethanolic extract of fresh plant *Eichhornia crassipes* were compared with ascorbic acid for the reduction of the Fe^{3+}- Fe^{2+} transformation in the presence of the ethanolic extract of the plant. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The antioxidant activity has been reported to be concomitant with the development of reducing power. The presence of reductant (antioxidants) in the extracts causes the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form. Therefore Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 680 nm [11, 13]. The reducing power of ethanolic extract of the fresh plant *Eichhornia crassipes* increased with the increasing amount of sample. The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of the extract. All the concentration of ethanolic extract of the fresh plant *Eichhornia crassipes* showed significant activities when compared to standard ascorbic acid.

The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation [14].

The data presented in figure 2 and 3 indicate that the marked antioxidant activity of ethanolic extract is due to presence of reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction.

EC50 value of ethanolic extract is 28µg (Figure 1) and that of ascorbic acid is 270µg (Figure 3) respectively. This shows that the reductones present in 300µg of ascorbic acid was found to be in 30µg of the ethanol extract.

Reducing power assay was also carried out for the fresh plant *Eichhornia crassipes* by considering the time as variant. It is quite obvious from the results that the reducing power of ethanol extract increases with increasing concentration and 50µg/ml of extract gave 0.67 absorbance at 680 nm, comparable with ≈400 µg/ml of standard (ascorbic acid) absorbance (Figure 2 and 3). With variation in time, absorbance increased. Maximum absorbance (0.88) was noted with 10µg/ml of the extract in 30 min which was comparable to the activity of 500µg/ml of ascorbic acid. Hence the reducing capacity of ethanolic extract of the fresh plant *Eichhornia crassipes* was found to be significant indicator of its potential antioxidant activity. This may be due to the presence of phytochemicals including flavonoids, phenolics which are responsible for antioxidant activity.

Figure 1: Reducing power assay of ethanolic extract of waterhyacinth with concentration variation

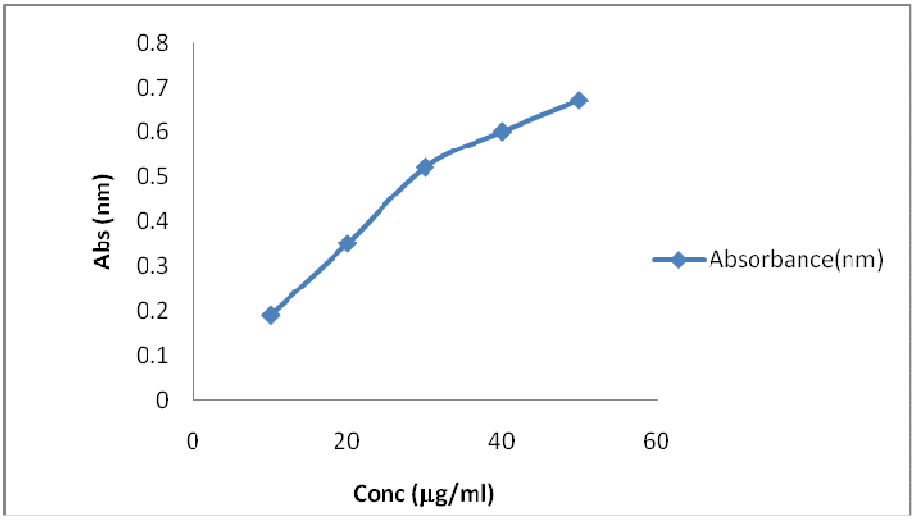
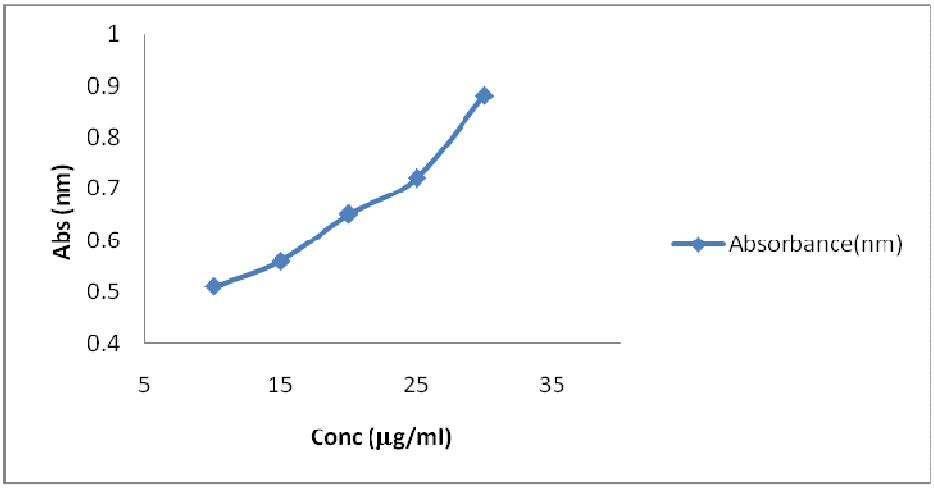


Figure 2: Variation of reducing power assay of ethanolic extract of waterhyacinth with Time



Reducing power assay of ethanol fractionate of dry *Eichhornia crassipes* (Mart.) Solms at different concentrations and time delay was evaluated by Jayanthi *et al* [15] showed that the absorbance increased with time and concentration.

The reducing power of petroleum ether, ethyl acetate, acetone and hydrolysed extract of waterhyacinth determined by ferricyanide method showed that the extracts of waterhyacinth possess good antioxidant activity [7].

DPPH Assay

Free radical scavenging potential of ethanol extract and ascorbic acid at different concentrations was tested by DPPH method. The results demonstrated good free radical scavenging activity of the extract.

The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude mixtures such as the ethanolic extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 520 nm. Radical scavenging activity increases with increasing percentage of the free radical inhibition. DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants

towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up [11]. From the present results, it may be postulated that ethanolic extract of the fresh plant *Eichhornia crassipes* reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles.

Figure 3: Variation of reducing power assay of Ascorbic acid

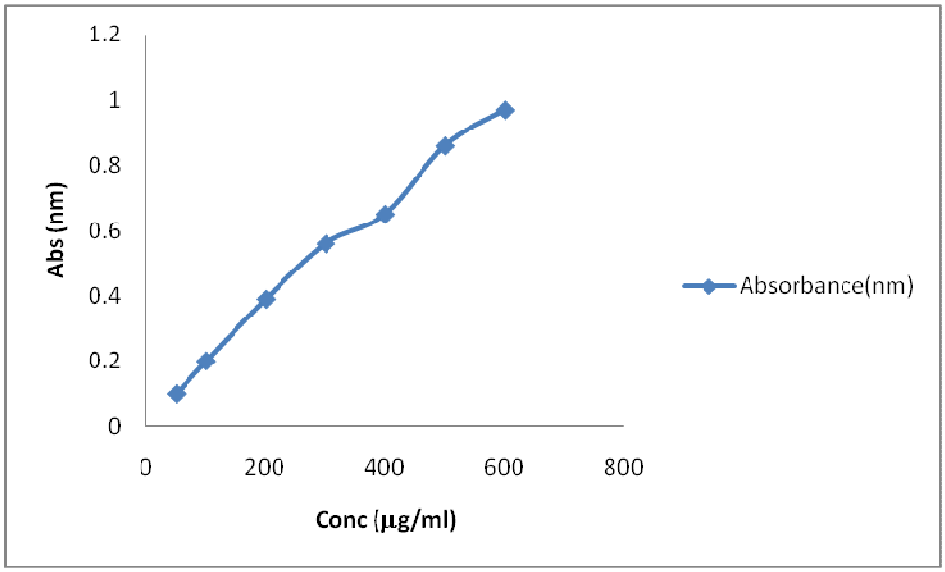


Figure 5 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in the ethanolic extracts of the fresh plant *Eichhornia crassipes* and the standard ascorbic acid, as a reference compound, presented the highest activity at all concentrations. The graph presented here indicated that the IC50 values were found to be 29µg and 400µg of ethanolic extract of the fresh plant *Eichhornia crassipes* and ascorbic acid respectively. Therefore 29µg of the ethanol extract is comparable with the activity of 400µg of ascorbic acid.

Figure 4: Effect of ascorbic acid on DPPH assay

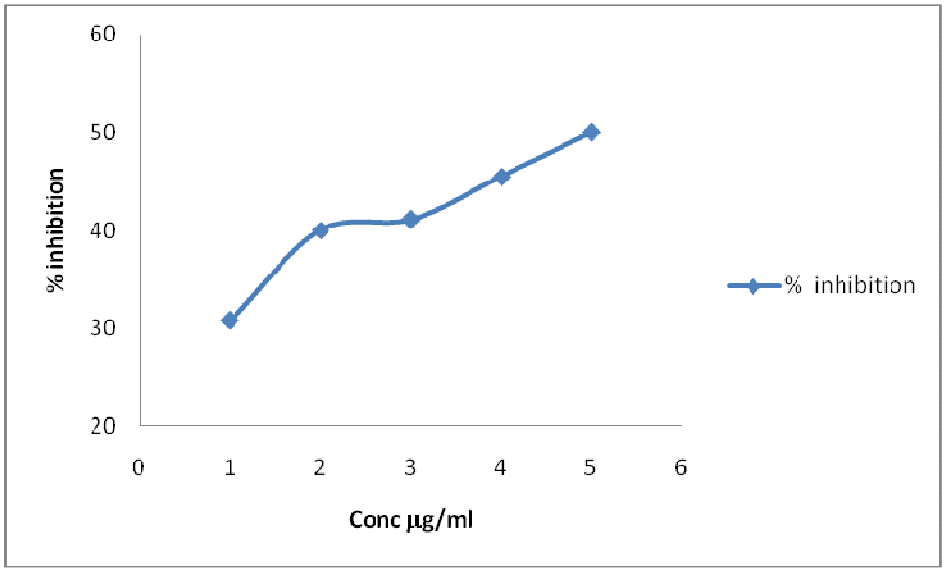
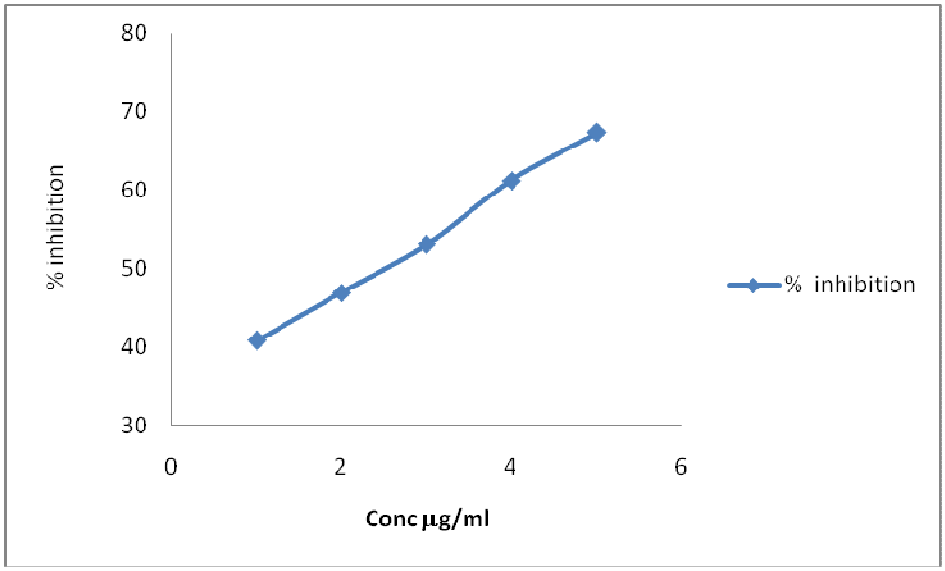


Figure 5: Antioxidant activity of Ethanol extract of waterhyacinth- DPPH assay



It is well evident from the results (Figure 5) that the free radical scavenging activity of the ethanol extract of waterhyacinth increases with increase in the concentration of the extract. Nearly 67% inhibition was noted with just 50 µg/ml of extract which shows the good antioxidant potential of the world's worst weed.

The DPPH scavenging assay of the petroleum ether, acetone, ethyl acetate, aqueous, hydrolysed extracts and fractionates viz the ethanol, the aqueous fractionate, methanol fractionate and the aqueous fractionate of dry waterhyacinth evaluated by Jayanthi *et al* [16] showed that the hydrolysed extract has good DPPH scavenging activity.

CONCLUSION

Eichhornia crassipes, a water succulent plant even when fresh shows good antioxidant activity. This is an indication that the plant can be harvested and processed fresh.

Acknowledgement
The authors thank the authorities of the Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore-43 for providing the facilities to carry out this research work.

REFERENCES

[1] Lokesh Deb, Ravi Gupt, Amit Sankar Dutta, Akhilesh Yadav, Debjit Bhowmik, K.P.Sampath Kumar, *Der Chemica Sinica.*, **2010,** 1(3), 157-164.
[2] S. Vidyadhar, T. Sheela, L. Shiva Kumar reddy, T. K. Gopal, D. Chamundeeswari, A. Saidulu, C. UmaMaheswara reddy, *Der Pharmacia Lettre*, **2010,** 2(6), 252-256.
[3] P. Arulpriya, P. Lalitha, S. Hemalatha, *Der Chemica Sinica.*, **2010,** 1(2), 73-79.
[4] Sajeeth, P. K. Manna, R. Manavalan, *Der Chemica Sinica.*, **2010,** 2(2), 220-226.
[5] P. Arulpriya, P. Lalitha, S. Hemalatha, *Der Chemica Sinica.*, **2010,** 1(3), 23-32.
[6] Sudhakar Kommu, Vijaya laxmi Chiluka, N. L. Gowri Shankar, L. Matsyagir, M. Shankar, S. Sandhy, *Der Chemica Sinica.*, 2011, 2(3), 193-199.
[7] P. Jayanthi and P. Lalitha, *Inter. J. Pharm. Sci.*, **2011**, 3(3), 126-128.
[8] A. K. Asha, C. T. Rasika, R. D. Nirmala, P. S. Jyoti, *Ann. Biol. Res.*, **2011**, 2 (1) ,176-180.
[9] A. Malik, *Environ. Int.*, **2007**, 33, 122.
[10] P. Jayanthi, P. Lalitha, K. S. Shubashini, *J. Pharm. Res.*, **2011**, 4 (5), 1405-1406.
[11] F. Nikhat, D. Satynarayana, E. V. S. Subhramanyam, *Asian J. Research Chem.*, **2009**, 2 (2), 218-221.

[12] M. Oyaizu, *Jpn. J. Nutr.*, **1986,** 44, 307-314.
[13] S. Gowri Shyamala, K. Vasantha, *American-Eurasian J. Sci. Res*, **2010**, 5 (2), 114-119.
[14] G. K. Jayaprakasha, Tamil Selvi, K. K. Sakariah, *Food Res. Inter.*, **2003**, 36, 117–122.
[15] P. Jayanthi, P. Lalitha, *J. Pharm. Res*., **2011**, 4, 4003-4005.
[16] P. Jayanthi, P. Lalitha, *J. Pharm. Res*., Accepted for publication.