P.Jayanthi et al. / Journal of Pharmacy Research 2012,5(2),946-948

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

DPPH scavenging assay of the solvent extracts and fractionates of *Eichhornia crassipes* (Mart.) Solms

P.Jayanthi* and P.Lalitha

Research Scholar,Department of Chemistry,Avinashilingam Institute for Home Science and Higher Education for Women ,Coimbatore-641 043,Tamilnadu,India

Assistant Professor (SS),Department of Chemistry,Avinashilingam Institute for Home Science and Higher Education for Women ,Coimbatore-641 043,Tamilnadu,India

Received on:10-11-2011; Revised on: 15-12-2011; Accepted on:12-01-2012

ABSTRACT

The antioxidant activity of the petroleum ether(PE), acetone(Ac), ethyl acetate(EA), aqueous(Aq), hydrolysed (Hy) extracts and fractionates viz the ethanol (EFA), the aqueous fractionate (AFE), methanol fractionate (MFA) and the aqueous fractionate (AMF) of *Eichhornia crassipes* (Mart.) Solms was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay. Of all the extracts and fractionates, hydrolysed extract showed better free radical scavenging activity. The extracts and the fractionates showed scavenging activity better than that of the standard ascorbic acid (AA). The results obtained depicts that the waste plant waterhyacinth which is considered to be a major threat to the environment and economy possess good antioxidant activity and this plant can be exploited for use as an effective natural antioxidant.

Key words:*Eichhornia crassipes*, antioxidant activity, DPPH, phytochemicals

INTRODUCTION

Physiological and biochemical processes of living cells may result in the generation of free radicals and other reactive oxygen species as by-products. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, ageing, and other degenerative disease in humans [1]. These free radicals have aroused significant interest among the scientific community because of the various pathological conditions that it causes. Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide [15]. Petroleum ether, ethyl acetate, acetone and hydrolysed extracts of waterhyacinth show good reducing power in ferric reducing assay [5]. In continuation of our studies on antioxidant activity, we now report the DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability of *Eichhornia crassipes*.

MATERIALS AND METHODS

Chemicals:

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) in free radical form was obtained from Sigma Chemical Co. All solvents used in the study were of laboratory grade and was purchased from SD fine.

Plant collection:

Waterhyacinth is an easily accessible plant and was collected from Singanallur boat house, Coimbatore, Tamilnadu. 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 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:

Waterhyacinth (1.5kg) 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.

Waterhyacinth (20g) was extracted successively with ethyl acetate (250mL), water (250mL) twice for 6 hours and desolvetised. The aqueous extract was fractionated with ethanol and methanol respectively yielding ethanol fractionate (EFA), aqueous fractionate of ethanol (AFE), methanol fractionate (MFA) and aqueous fractionate of methanol (AFM).

Waterhyacinth (320g) was extracted four times with chloroform (2.5 L) for 6 hours and desolvetised to obtain chloroform extract.

A small portion (1.5kg) of the plant residue was extracted with 1% hydrochloric acid (3L) for 6 hours to get hydrolysed extract.

Radical Scavenging activity using DPPH assay:

Principle

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

$$DPPH^{\bullet} + (H\text{-}A) \rightarrow DPPH + (H\text{-}A)$$

(Purple) **(Yellow)**

Antioxidants react with DPPH• which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance's decreased 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 used

Methanolic solution of DPPH (0.3 mM): 20 mg of DPPH was dissolved in 20ml of analytical grade methanol and ascorbic acid 1%.

Protocol

0.3 mM solution of DPPH 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. 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 absorbance of the reaction

***Corresponding author.**
P.Jayanthi*
Research Scholar,Department of Chemistry,Avinashilingam Deemed University for Women, Coimbatore-641 043,Tamilnadu,India

mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH• radical was calculated using the following equation:

$$\text{DPPH Scavenged}(\%) = \left(\frac{A_{control} - A_{test}}{A_{control}} \right) * 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extracts and the fractionates were expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50 [12]

RESULTS AND DISCUSSION:

DPPH• is a widely used free radical to find the scavenging ability of the antioxidants present in an extract [3,7,17]. DPPH• is a stable free radical in methanolic solution. In its oxidized form, the DPPH• radical has an absorbance maximum centered at about 520 nm [11]. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity [8]. Hence this assay was chosen among other methods to determine the scavenging ability of the extracts/fractionates of waterhyacinth.

DPPH• is a stable free radical and readily accepts an electron or hydrogen radical to become a stable diamagnetic molecule ((2,2-diphenyl-1-picrylhydrazine). The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Hence, DPPH• is used as a substrate to evaluate antioxidative capacity of antioxidants [13]. Radical scavenging activity increases with increasing percentage of the free radical inhibition.

Leaves of waterhyacinth contain antioxidizing agents and glutathione, and this antioxidant was determined by an enzymatic assay using glutathione reductase [4]. Methanol leaf extract [2] and ethanol extract of waterhyacinth shows good DPPH• radical scavenging ability and ethanol extract of waterhyacinth shows good reducing capacity [9].

The percentage inhibition of the extracts and fractionates of waterhyacinth are given in Table 1. The extracts and fractionates show a dose response relationship and it showed a better scavenging activity than the standard ascorbic acid. The results indicate that the extracts / fractionates of waterhyacinth contains compounds responsible for scavenging the radical. Phenols, flavonoids [14, 16], tannins [16] alkaloids, glycosides [14] are good antioxidant substances and prevent or control oxidative stress related disorders. Waterhyacinth contains flavonoids, alkaloids, tannins, glycosides and phenols [6].

Hydrolysed extract of waterhyacinth showed maximum inhibition percentage than other extracts/ fractionates. This is because hydrolysis liberates bound antioxidant substances bound to sugars [16]. It is obvious from the table that acetone extract (Ac) (75%) showed maximum inhibition than the chloroform extract (71%). Albeit aqueous extract contains alkaloids, flavonoids, and other phytochemicals [6], it exhibited 69% inhibition at 15 µg/ml. This might be because of the interaction between the phytochemicals that render the hydrogen atom unavailable for the DPPH• radical.

The fractionates viz AFM, AFE, EFA and MFA show lesser inhibition percentage than the aqueous extract (AQE). Synergistic effect existing between the antioxidants in the aqueous extract may be the reason for the higher inhibition percentage of the aqueous extract of waterhyacinth. The IC_{50} values for the extracts and fractionates are given in Table 1.

This study affirms the DPPH• scavenging ability of the extracts and fractions of waterhyacinth, with the results comparable to those of the standard ascorbic acid. Further studies may be carried out to isolate the compounds responsible for the antioxidative characteristics of the plant extracts and fractionates and to find out the mechanism of action of these antioxidants with the free radicals.

Table 1. Antioxidant activity of the extracts and fractionates

Extractants	Regression equation	r^2	Maximum inhibition percentage (%)	IC_{50} (µL/mL)
Hy	y = 4.5667x - 1.5	0.9689	71	5.4
Ac	y = 4.5x + 7.3	0.9478	75	9.48
PE	y = 4.7x - 1.5	0.9478	68	10.9
AQE	y = 4.6x - 1.8	0.9923	69	11.26
$CHCl_3$	y = 4.5667x - 1.5	0.9689	71	11.27
EA	y = 2.1333x + 21.4	0.9961	54	13.4
MFA	y = 3.2667x + 14.4	0.939	59	10.89
EFA	y = 4.0333x + 0.1	0.9537	63	12.37
AFM	y = 3.9667x - 4.5	0.9628	53	13.73
AFE	y = 2.4667x + 16	0.9695	54	13.78
Ascorbic acid	y = 1.6429x + 26.286	0.9716	63	14.43

ACKNOWLEDGEMENT:

The authors thank Avinashilingam Institute for Home Science and Higher Education for Women for providing necessary facilities to carry out this work.

REFERENCES:

1. Aiyegoro OA, Okoh AI, Preliminary phytochemical screening and *invitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC, BMC Complementary and Alternative Medicine, 10, 2010, 1-8.
2. Ali H, Ganesh N, Ahi JD, Antioxidant activities of *Eichhornia crassipes* (waterhyacinth) leaf extract by the DPPH free radical method: The World's worst aquatic plant**.** J of Ultrachemistry, 5, 2009.
3. Beknal AK, Prakash GK, Halkai MA, Kulkarni U, Patil B S, Soodam SR.. Phytochemical investigation and antioxidant activity study of *Drynaria quercifolia* Linn. Rhizome, Int J of Curr Pharm Res, 2, 2010, 36-39.
4. Bodo R, Azzouz A, Hausler R, Antioxidative activity of waterhyacinth components. Plant Science, 166, 2004, 893–899.
5. Jayanthi P, Lalitha P, Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Int J of Pharm and Pharm Sci, 3,2011, 126-128.
6. Jayanthi P, Lalitha P, Phytochemical investigation of the extracts and the solvent fractionates of the aqueous extract of *Eichhornia crassipes*. J of Pharm Res, 2011, Accepted for publication.
7. Katalinic V, Milos M, Modun D, Music I, Boban M, Antioxidant effectiveness of selected wines in comparison with (+)-catechin. Food Chem, 8, 2004, 593–600.
8. Koleva II, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal, 13, 2001,8–17.
9. Liu CC, Zhao GL, Li YN, Ding ZP, Liu QG, Li JL, Contribution of phenolics and flavonoids to anti-oxidant activity and of ethanol extract from *Eichhornia crassipes*. Adv Mater Res, 156 – 157, 2010, 1372-1377.
10. Marxen K, Vanselow KH, Lippemeier S, Hintze, Ruser A, Hansen UP, Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. Sensors **7**, 2007, 2080-2095.
11. Molyneux P, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26, 2004, 211–219.
12. Nikhat F, Satynarayana D, Subhramanyam EVS, Isolation, characterisation and screening of antioxidant activity of the roots of *Syzygium cuminii* (L) Skeel. Asian J Research Chem 2, 2009, 218-22.
13. Oktay M, Gulcin I, Kufrevioglu OI, Determination of *invitro* anti

oxidant activity of fennel *(Foeniculum vulgare)* seed extracts. Lebensum-Wiss U-Technol 36, 2003, 263-271.

14. Pandey S, Sah SP,Sah ML, Mishra D, An antioxidant potential of hydromethanolic extract of *Urtica parviflora* Roxb. J Basic and Clin Pharm 1, 2010, 191-195.
15. Parthasarathy S, Azizi JB, Ramanathan S, Ismail S, Sasidharan S, Said MIM, Mansor SM, Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from *Mitragyna Speciosa* (Rubiaceae Family) leaves, Molecules, 14, 2009, 3964-3974.
16. Prosper-Cabral NB, Agbor GA, Oben JE, Ngogang JY, Phytochemical studies and antioxidant properties of four medicinal plants used in Camerron. Afr.J.trad.CAM, 4, 2007, 495-500
17. Šeruga M, Novak I, Jakobek L, Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chem, 124, 2011, 1208–1216.

Source of support: Nil, Conflict of interest: None Declared