



PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES OF *EICHHORNIA CRASSIPES* (MART.) SOLMS

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Article Received on 09/06/2020

Article Revised on 29/06/2020

Article Accepted on 19/07/2020

ABSTRACT

Eichhornia crassipes (Water hyacinth) is listed as world's worst aquatic plant. Water hyacinth is a wellspring of phytochemicals which have a medicinal function. In this study, the high degree of peroxidation and nonenzymatic antioxidant systems were detected in extracts. The phytochemical analysis of the extracts was performed using n-hexane, water, ethyl acetate and ethanol. The extracts showed the presence of primary and secondary metabolites that include carbohydrate, protein, alkaloids, glycosides tannins and phenols. The antioxidant activity ethanolic extract was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay. The results obtained showed that the plant water hyacinth, in the world's worst weed which poses major threat to the environment and economy and could be utilized in an Efficient as a natural antioxidant.

KEYWORDS: *Eichhornia crassipes*, Phytochemical Analysis, DPPH, antioxidant activity.

INTRODUCTION

Eichhornia crassipes (Water hyacinth) belongs to family Pontederiaceae is free floating aquatic macrophyte. It is considered as a most productive plant on earth and listed in world's ten worst aquatic plant.^[1] It is a source of many compounds such as alkaloids, glycosides, tannins, phenolic acid and other metabolites with radical scavenging activity.^[2] The utilization of water hyacinth is done due to unsuccessful methods to control its growth. It improves the hydro-physical and chemical parameters and supplies various nutrients to soil. So it can also be used as co-compost. It can be the source of natural antioxidants for retarding lipids peroxidation.^[3]

Free radical induced oxidative damage has long been thought to be the most important cause of many diseases such as diabetes, stroke, cancer, arteriosclerosis, and cardiovascular diseases.^[4,5] Antioxidant compounds inhibit oxidation.

They produce free radicals which can damage cell and also it protects the cell by inhibiting the initiation or propagation of oxidative chain reaction. Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms.^[6] Antioxidants exhibit their antioxidant activity either by inhibiting lipid peroxidation, by scavenging free radicals and active oxygen species, preventing the decomposition

of hydrogen peroxides into free radicals or by chelating heavy metal ions.^[7,8]

The main aim of present study were to carry out the phytochemical screening from petioles, leaves, flowers of *Eichhornia crassipes* in solvents like ethanol, ethyl acetate, water, n-hexane and to evaluate antioxidant activity by using DPPH(2,2-diphenyl -1-picrylhydrazyl). Its growth depends on the ecological factors. It is observed that its growth is faster at summer season between temperature 20-30⁰C and less growth is observed in winter season between temperature 8-15⁰C.^[9] The information obtained can help to understand the antioxidant compounds present in these aquatic herbs to improve their potential application as natural antioxidants in pharmaceutical and functional foods that promote health.

MATERIALS AND METHODS

Collection of plant material

The whole plant material (roots, leaves, flowers and petioles) were collected from the Rankala, Kolhapur, India in the month of August. It was washed using distilled water. The plant material was dried under shadow. It is then granted to form a fine powder. The prepared powder was stored in air tight container at room temperature until further use.

Extract preparation

The extract was prepared by using solvents like n-hexane, water, ethyl acetate, and ethanol. The powder of 10gms was weighed and extracted for 24 hrs (percolation) and stirred meanwhile using solvent 100ml. Further it was filtered. Procedure was followed for all four solvent. The four extract were used further.

Phytochemical screening^[10-13]

Test for carbohydrate

Molisch's test: Molisch's reagent was added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two- three minutes and then diluted with 5 ml of distilled water. Formation of a red or violet colour in between two layers indicate positive test.

Fehling's test: About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for steroids

Salkowski reaction: To 2ml extract, add 2ml chloroform and 2ml concentrate H₂SO₄. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Lieberman-Burchard reaction: Mix 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops concentration H₂SO₄ from side of test tube. First red, then blue and finally green colour appears. The procedure was repeated for four extract.

Test for saponin glycosides

The presence of saponin is detected by using foam test. Take the extract in test tube and shake vigorously. Foam is observed at the top of extract.

Test for Tannins and phenolic compounds

- Lead acetate solution was added to all extract and observation was generation of white ppt.
- Acetic acid solution was added to all extract and observation was red colour solution.
- Dilute HNO₃ was added and observation was reddish to yellow colour formation.

Test for cardiac glycosides

Keller killiani test- Take extract and add equal volume of water and lead acetate solution. Shake and filter it. Take the filtrate extracted with equal volume of chloroform. Chloroform extract evaporated to dryness and dried residue is dissolved in glacial acetic acid, few drops of ferric chloride were added. Sulphuric acid is added. Reddish brown layer observed.

Test for cardiac flavonoid

For the confirmation of flavonoid in the selected plants, 0.5 g of each selected plant extract was added in a test tube with 10 ml of distill water, 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of 1ml concentrated H₂SO₄. Indication of yellow colour showed the presence of flavonoid in each extract

Test for alkaloids

0.2 g of the selected plant samples was added in each test in the qualitative analysis of the phytochemicals, the plant extract tested indicated presence of different compounds. It is an indication of the presence of a few secondary metabolites in the plant *Eichhornia crassipes*.

DPPH assay (2,2-diphenyl -1-picrylhydrazyl)^[14-17]

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-bpicrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

The ability of Compounds to scavenge DPPH radical was assessed using Ramanathan Sambath Kumar et al and Manzocco et al., 1998 method with modification. Briefly, 1 ml of *Eichhornia crassipes* extract (1000 µg/ml) was mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resultant absorbance was recorded at 517 nm after 30 min. incubation at 37°C. The percentage of scavenging activity was derived using the following formula,

$$\text{Percentage of inhibition (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where A control - absorbance of DPPH

A sample - absorbance reaction mixture (DPPH with Sample)

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of *Eichhornia crassipes* was studied by using various chemical tests. The various extract was used to determine the phytoconstituents. The phytoconstituents present in *Eichhornia crassipes* are saponins, tannins, phenolic compounds, sterols, cardiac glycoside.

Table 1: Phytochemical screening of *Echhornia crassipes* in various extract.

Phytoconstituents	n-hexane	Ethanol	Water	Ethyl acetate
Carbohydrate	+	+	+	+
Steroids	+	+	+	+
Saponins	+	+	+	+
Flavonoids	-	-	-	-
Alkaloids	+	-	+	-
Tannins and phenolic compounds	+	+	+	+
Cardiac glycosides	-	-	+	-

DPPH radical scavenging assay

The DPPH scavenging activity was performed for testing antioxidant activity of ethanolic extract of *Eichhornia crassipes*. The hydrogen donating ability is responsible for the effect of antioxidant on DPPH. The DPPH is

stable free radical and accept an electron or hydrogen radical to become stable diamagnetic molecule. The ethanolic extract is allowed to react with stable free radical. The antioxidant activity of *Eichhornia crassipes* by DPPH assay found their scavenging activity.

Table 2: In-vitro antioxidant activity of *Eichhornia crassipes*.

Sample code	DPPH radical scavenging activity	
	Absorbance at 517nm	% inhibition
standard Ascorbic acid (1000 µg/ml)	0.36	63.26
Ethanol extract 1000 µg/ml	0.51	47.95

The result shows to provide rich source of natural bioactive compounds with antioxidant activities. It suggested that *Eichhornia crassipes* was potential source of antioxidant activity and can be used as preservative in food and non-food system. It shows 47.95% inhibition according to the DPPH scavenging activity.

CONCLUSION

It is concluded that the investigated plant *Eichhornia crassipes* showed very good antioxidant activity for DPPH assay. The plant showed presence of various phytoconstituent like carbohydrate, alkaloids, sterols, saponins, cardiac glycoside, phenolic and flavonoidal content. This piece of work gives idea about further research on the antioxidant ability of the plant use as a commercial medicine as a natural antioxidant for the free radicals related diseases.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. H. N. More, Principal, Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing facilities to carry out the work.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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