

Research Article



HPLC Detection of Glutathione in the Ethyl Acetate and Aqueous Extract of *Eichhornia crassipes* (Mart.) Solms

P. Lalitha*, Shubashini K Sripathi, P. Jayanthi

Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India.

*Corresponding author's E-mail: goldenlalitha@gmail.com

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ABSTRACT

Glutathione, a ubiquitous thiol-containing tripeptide is a low-molecular weight, water soluble antioxidant. It plays a major role in the removal of reactive species. *Eichhornia crassipes* is an aquatic weed possessing pharmacological potential and is a source of many antioxidants. HPLC established glutathione detection in *E. crassipes* was carried out with a PDA detector. The study revealed the presence of glutathione in the ethyl acetate and aqueous extracts of *E. crassipes*. This paper further confirms the antioxidative capacity of the extracts of this plant.

Keywords: *Eichhornia crassipes*, antioxidant, glutathione, HPLC, water hyacinth.

INTRODUCTION

The tripeptide, γ -L-glutamyl-L-cysteinyl-glycine widely known as glutathione (GSH) is a sulphhydryl, cellular hydrophilic antioxidant, antiotoxin and enzyme cofactor that detoxifies reactive oxygen species (ROS).¹⁻³ ROS are normal by-products of aerobic metabolism, but can cause lipid peroxidation, protein damage, and eventually cell death if allowed to persist. In detoxifying these ROS, glutathione (GSH) is oxidized to glutathione disulfide (GSSG). *In vivo*, GSSG is then regenerated by a series of enzymatic reactions termed the ascorbate–glutathione or Halliwell–Asada pathway, which ultimately depends on photosynthetically derived NADPH as a source of reductant. Furthermore, glutathione enhances the functional ability of other crucial antioxidants vitamin E and C.⁴ In addition to these antioxidant functions, GSH has been implicated in many aspects of plant development, including cell division, regulation of growth, cellular differentiation, stress signalling,³ reduction of ribonucleotides to deoxyribonucleotides, protein and gene regulation via thiol:disulphide exchange reactions.⁵ The importance of GSH is obvious by its extensive efficacy in plants, mammals, fungi and some prokaryotic organisms.

The ratio of GSH and GSSG is useful to analyze oxidative stress and disease risk.⁶ GSH has long been detected by numerous analytical methods. Particularly High Performance Liquid Chromatography (HPLC)⁷ with various detection techniques, such as ultraviolet (UV) absorbance and fluorescence (FL) detection, is widely used because of its convenience, specificity and satisfactory sensitivity. Use of HPLC in detection of glutathione has been prominent owing to its accuracy⁶ and ease of detection compared to other techniques.

Eichhornia crassipes (waterhyacinth) is an aquatic weed known for its rapid growth and proliferation. This plant is spread almost in all parts of the world. Threats posed by

the plant to the environment have instigated researchers to look for various avenues of effective use of the plant. The plant is reported to possess several pharmacological activities.⁸⁻¹¹ *E. crassipes* is supposed to contain high amounts of cysteine-based proteins like metallothioneins which scavenges free radicals. *E. crassipes* contains antioxidants including glutathione and the plant displays activity similar to that of soy and garlic.¹² Detection of glutathione in the protein mass extracted from *E. crassipes* was carried out by HPLC with pure glutathione as the internal standard.¹³ This paper focuses on the determination of glutathione in the ethyl acetate and aqueous extract of *E. crassipes* by HPLC and to establish the importance of this weed with a view to convert the desecrated plant material to a commercially significant resource.

MATERIALS AND METHODS

Plant collection

E. crassipes was collected from a local water body at Coimbatore, Tamil Nadu and the plant sample was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore- 641 002 with the voucher specimen number BSI/SRC/5/23/2011-12/Tech.

Extraction of the plant material

The root portion was cut off, cleaned and shade dried for 20 days. The dried plant material was segmented well and ground. *E. crassipes* (100 g) was extracted by percolation method successively with ethyl acetate (2000 mL) and water (1000 mL) twice for 6 h. The extracts were desolvated to give ethyl acetate extract and aqueous extract.

Instrument

A Shimadzu HPLC system equipped with Class VP series version 6.1 software with photo diode array detector



(SPD M10A VP), CTO-10AS VP column oven, CBM- 10A VP system controller and reverse phase C18 phenomenex column (250 mm × 4.6 mm) was used for the analysis.

Sample preparation

The ethyl acetate extract (10 mg) of *E. crassipes* was dissolved in 10mL HPLC grade methanol. The aqueous extract (200 mg) was dissolved in 8 mL HPLC grade water. A stock solution of reduced glutathione 100ppm was prepared with HPLC grade water and filtered using SRP 15 syringe of 0.20 µm diameter filter (filter area of 1.7 cm²) for rapid ultra-cleaning of the sample. The sample (1mL) was injected into the column for each analysis.

Chromatographic conditions

The mobile phase consisted of two solvent systems (A: 0.06% trifluoroacetic acid (TFA) in water (v/v) and B: 100% acetonitrile) in a gradient (50% A and 50% B). The column temperature was maintained at 0°C, flow rate at 1.0 mL/min and run time was set as 40 minutes. The detection of glutathione was carried out at 225 nm using PDA detector.

Determination of reduced glutathione

The method of Moron *et al* (1979) was adopted for the determination of reduced glutathione.¹⁴ Aqueous leaf extract (200 µL) was diluted with 800 µL distilled water and treated with sodium phosphate EDTA buffer containing 0.6 mol/L 5,5'-dithiobis (2-nitro benzoic acid) reagent. The colour developed was read at 412 nm against the reagent blank. A calibration curve was obtained with different concentrations (25,50,75,100 and 125µg) of standard glutathione.

RESULTS AND DISCUSSION

The ethyl acetate and aqueous extracts of *E. crassipes* obtained by percolation method was analysed for the presence of glutathione using HPLC. The composition of the mobile phase was optimized by varying the concentration of the two mobile phases. The composition 0.06 % trifluoroacetic acid (TFA) in water (v/v) (A) and 100 % acetonitrile (B) (50% A and 50% B) was chosen for the

study.¹³ The HPLC of standard glutathione, ethyl acetate extract, and aqueous extract was recorded in the optimized chromatographic conditions. Glutathione showed two peaks at Rt 0.37 and 2.25 min. Varying concentrations of the ethyl acetate extract in methanol (1 mg, 1.25 mg, 1.6 mg) were analysed. The ethyl acetate extract showed three peaks at Rt 1.35, 2.52 and 2.91 min (Figure 1). The area of the peak at Rt 2.9 min was found to increase with increase in concentration whereas the other peaks showed a decrease in area with increase in concentration indicating the predominancy of the compound. In comparison with the standard, the peak at Rt 2.9 min was assigned to glutathione. The aqueous extract showed the presence of three peaks at Rt 2.21, 2.49 and 2.96 min (Figure 2). The area of the peaks was found to increase with the increase in concentration. The peak at Rt 2.96 min was assigned to glutathione in comparison with the standard. The drift in the Rt from 2.21 to 2.9 for the extract might be due to change in mobile phase composition caused when pre-mixed mobile phases lose organic solvent through evaporation as the run progresses. Trifluoroacetic acid might have been lost to the headspace above the eluent and would have caused a progressive change in retention time during the analysis.¹⁵ Estimation of reduced glutathione by the method of Moron *et al* (1979) indicated the optical density of the extract at 412 nm to be 0.17 and 0.15. The results of the study reveal 1g of *E. crassipes* leaves to contain 0.1 mmole of reduced glutathione.

The presence of glutathione in the dry leaves of *E. crassipes* has been detected by HPLC and estimated by total glutathione assay.^{12,13} Glutathione plays a significant role in maintaining the protein –SH groups in the reduced state and in removing toxic peroxides formed in metabolism.¹² In the present study, presence of glutathione was ascertained in the ethyl acetate and aqueous extracts of *E. crassipes*. This may provide an opportunity for the plant to be used in pharmacological and cosmeceutical industry since glutathione has been reported to possess strong antioxidant and antiageing activity.

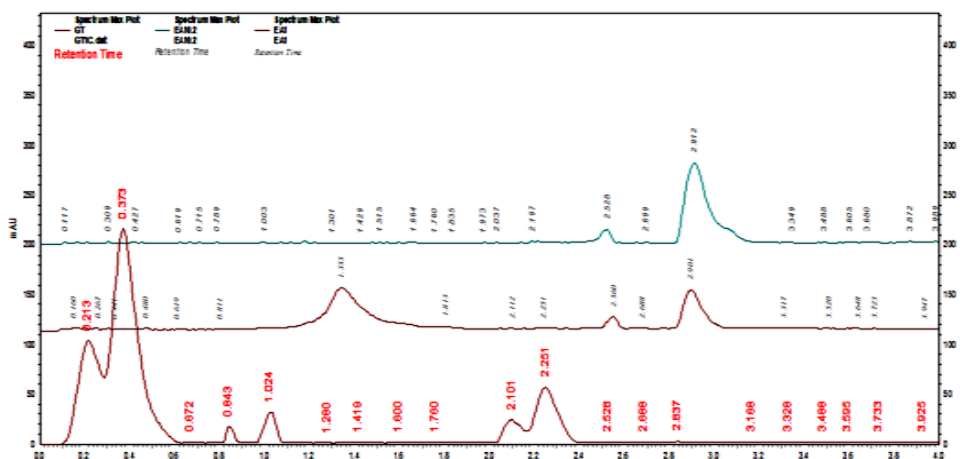


Figure 1: HPLC of the ethyl acetate extract of *E. crassipes*

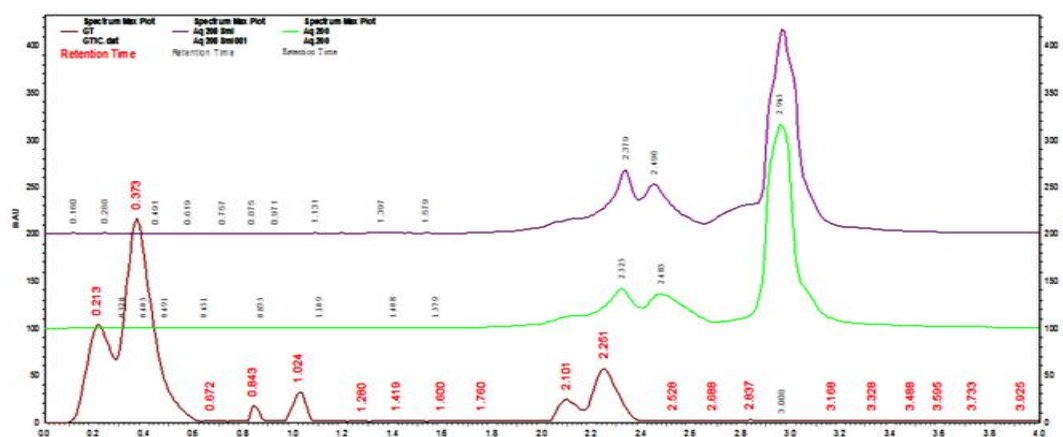


Figure 2: HPLC of the aqueous extract of *E. crassipes*

CONCLUSION

HPLC analysis of the ethyl acetate and aqueous extracts of *E. crassipes* shows the plant to contain glutathione. The concentration of glutathione was found to increase with increasing extract concentration. This study lends lead in the usefulness of the plant in pharmaceutical and cosmeceutical industry. Further work involving the isolation of glutathione from *E. crassipes* may enhance the usefulness of the plant as it is available in plenty throughout the world.

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