Antioxidant Activity and Detection of Glutathione in Flower Extract of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms.

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Abstract

Eichhornia crassipes (Mart.) Solms. (water hyacinth) is invasive weed aquatic plant that causes serious issues for rivers, lakes, and other reservoirs around the world, although it can be an excellent source for bioactive compounds. In this study, water hyacinth samples were collected from Dagon Township in Yangon. The extracts of aqueous, ethanol and methanol from the part of flowers were analyzed. The present study was carried out antioxidant activity using DPPH free radical scavenging assay and total phenolic contents in Gallic acid Equivalent (GAE) per gram as well as total flavonoid contents in Quercetin Equivalent (QE) per gram using spectrophotometric methods from aqueous, ethanol and methanol extracts of flower *Eichhornia crassipes* (Mart.) Solms. (water hyacinth) (Pontederiaceae). Due to HPLC analysis, it was found that the aqueous, ethanol, methanol extracts water hyacinth flower showed the plant to contain glutathione assessed by total glutathione assay. Although these extracts showed antioxidant activity, aqueous extract was the most potent. Methanol extract revealed more inhibitory percentage of anti-diabetes and the ethanol extract water hyacinth showed more inhibitory percentage of achE than the other extracts. Maximum amount of total phenolic and flavonoid compounds were observed in aqueous extract and minimum in ethanol extract of water hyacinth.

Introduction

Eichhornia crassipes (Mart.) Solms., commonly known as water hyacinth is warm water aquatic plant belonging to the family of Pontederiaceae. Water hyacinth is listed as one of the most productive plants on earth and is considered the world's worst aquatic weed. The "beautiful blue devil" water hyacinth, recognized by its lavender flower and shining bright leaves which spread at an alarming rate. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Phytochemicals are present in a variety of plant utilized as important components of both human and animal diets. Plant products are rich sources of phytochemicals which have been found to possess variety of biological activities including antioxidant, cytotoxic, and hepatoprotective potential. They act as reducing agents and revere oxidation by donating electrons and / or hydrogen ions.

The present study included : to extracts and quantity the phenolic, flavonoid from the flower of water hyacinth and to evaluate the antioxidant capacity of the extract of aqueous, ethanol and methanol extract using DPPH(2, 2 dipheny-1 pyreyl hydrazyl) radical scavenging ability. The information gained may help in understanding the antioxidant compounds present in these aquatic weeds to promote the potential application of these compound as natural antioxidants in foods, pharmaceuticals, and in health-promoting functional foods.

Materials and methods

Botanical Studies

The plants samples of *Eichhornia crassipes* (Mart.) Solms.(Water hyacinth) were collected from East Dagon Township,Yangon Region from February to October 2018. The fresh specimens of vegetative and reproductive parts were identified in

Botany Department of Dagon University with the help of available literatures such as Backer, (1963); Dassanayake, (1987) and Kress, (2003).

Chemical Studies

The samples were washed with water to remove impurities. After washing and cleaning, the sample was air dried and ground to get powdered and stored in air tight container. The chemical studies were carried out in Department of Oriental Medicine Resources, Jeonbuk National University, Republic of Korea from 3rd November to 30th November 2018. Firstly, the powdered sample was boiled with aqueous for 1 h, macerated with 95%ethnol and methanol for 12 h with the ratio of 1:10 (w/v). Secondly, the extracts were collected and filtered through Whatman No.1 filter paper and then filtered with nylon membrane paper. Finally, the aqueous, ethanolic and methanolic extracts were yield respectively. Then, these three crude extracts were observed antioxidant activity, anti-diabetes activity, AchE activity, total phenolic contents (TPC) and total flavonoid contents (TFC) by following methods shown in Figure 1, 2, 3, 4 and 5. In these experiment, the absorbance of these extract solution was measured at 517 nm by using UV spectrophotometer. Each experiment was done triplicate. This method is based on the reduction of colured free radical DPPH in ethanolic solution by different concentration of the samples. The anti-diabetic activity and Acetylcholineesterase (AchE) activity were measured at 405 nm besides TPC and TFC were recorded at 700 nm and 500 nm respectively. All data were resulted by calculation. Then, High Performance Liquid Chromatography (HPLC) analysis was also detected on these extracts of the selected sample. In the experiment, 1.5 ml (1500µL) of these extracts was used.

(i)Aqueous extract sample (40 µL)	+	DPPH Solvent (1800 µL)	+	Distilled water (160 µL)	kept in the dark for 30 mins →	UV check
(ii)Ethanol extract sample (40 µL)	+	DPPH Solvent (1800 µL)	+	EtOH (160 µL)	kept in the dark for 30 mins →	UV check
(iii)Methanol extract sample (40 µL)	+	DPPH Solvent (1800 µL)		MetOH (160 µL)	kept in the dark for 30 mins →	UV check

Figure 1. 2,2-Dipheny-1 picryl-hydrazyl (DPPH) Radical Scavenging Activity

(i)Aqueous extract sample(40µL)	+	α-glucosidase (200 µL)	kept for 10 mins at incubator37°C →	ρNPG 100 µL	kept for 20 mins at incubator37°C →	Na_2PO_3 (2000 µL)	→	UV check
(ii)Ethanol extract sample(40 µL)	+	α-glucosidase (200 µL)	kept for 10 mins at incubator37°C →	ρNPG 100 µL	kept for 20 mins at incubator37°C →	Na_2PO_3 (2000 µL)	→	UV check
(iii)Methanol Extract sample(40 µL)	+	α-glucosidase (200 µL)	kept for 10 mins at incubator37°C →	ρNPG 100 µL	kept for 20 mins at incubator37°C →	Na_2PO_3 (2000 µL)	→	UV check

Figure 2. Anti-diabetic activity

(i)Aqueous extract sample (150 µL)	+	Tri HCl buffer (1500 µL)	+	AchE solvent (150 µL)	Shaking for 10 mins at room tem: →	DTNB Solvent (150 µL)	+	Acetylthiochlorie (75 µL)	after 2mins →	UV check
(ii) Ethanol extract sample (150 µL)	+	Tri HCl buffer (1500 µL)	+	AchE solvent (150 µL)	Shaking for 10 mins at room tem: →	DTNB Solvent (150 µL)	+	Acetylthiochlorie (75 µL)	after 2mins →	UV check
(iii)Methanol extract sample (150 µL)	+	Tri HCl buffer (1500 µL)	+	AchE solvent (150 µL)	Shaking for 10 mins at room tem: →	DTNB Solvent (150 µL)	+	Acetylthiochlorie (75 µL)	after 2mins →	UV check

Figure 3. AchE activity (Acetylcholinesterase)

(i)Aqueous extract sample (100 µL)	+	2% Na_2CO_3 (2000µL)	wait for 3mins →	50% FCR Follin.Ciocalteau reagent (100 µL)	wait for 30mins →		**UV Check**
(ii)Ethanol extract sample (100 µL)	+	2% Na_2CO_3 (2000µL)	wait for 3mins →	50% FCR Follin.Ciocalteau reagent (100 µL)	wait for 30mins →		**UV Check**
(iii)Methanol extract sample (100 µL)	+	2% Na_2CO_3 (2000µL)	wait for 3mins →	50% FCR Follin.Ciocalteau reagent (100 µL)	wait for 30mins →		**UV Check**

Figure 4. Total Phenolic Content (TPC)

(i)Aqueous extract sample (250 µL)	+	Distilled water (1000 µL)	+	5% $NaNO_2$ (75 µL)	wait for 5mins →	10%$AlCl_3$. $6H_2O$ (150 µL	wait for 6mins →	1N NaOH (500 µL)	wait for11 mins →	UV check
(ii)Ethanol extract sample (250 µL)	+	Distilled water (1000 µL)	+	5% $NaNO_2$ (75 µL)	wait for 5mins →	10%$AlCl_3$. $6H_2O$ (150 µL	wait for 6mins →	1N NaOH (500 µL)	wait for11 mins →	UV check
(iii)Methanol extract sample (250 µL)	+	Distilled water (1000 µL)	+	5% $NaNO_2$ (75 µL)	wait for 5mins →	10%$AlCl_3$. $6H_2O$ (150 µL	wait for 6mins →	1N NaOH (500 µL)	wait for11 mins →	UV check

Figure 5. Total Flavonoid Content (TFC)

complete, bisexual, zygomorphic, trimerous, hypogynous; perianth 6, pale purple segments, the posterior segment largest with a bright yellow, lilac blue-bordered median blotch; stamens 6, 3 short anterior filament, 3 longer posterior one, filament long, anther dithecous, introrse, dorsifixed, longitudinal dehiscence; ovary superior, tricarpellary, syncarpous, trilocular, the placentation axile, α ovules in each locule in T.S. Fruits dry capsuled, pale brown, seeds many, black.

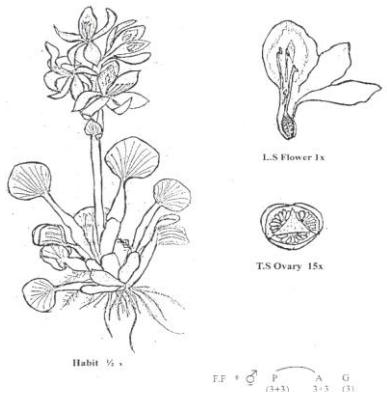


Figure 6. Diagram of *Eichhornia crassipes* (Mart.) Solms. Water hyacinth

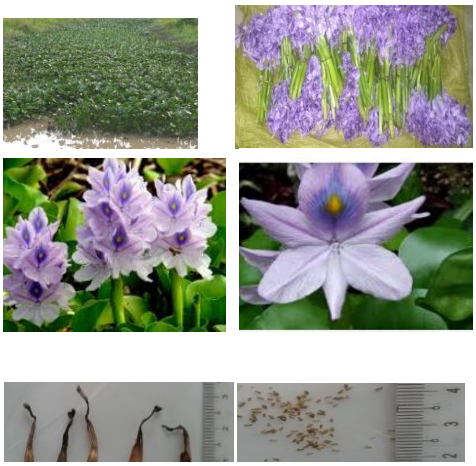


Figure 7. Morphological Characters of *Eichhornia crassipes* (Mart.) Solms. Water hyacinth

Chemical Studies

Antioxidant ability

From the obtained results, it was found that all extracts of flowers of water hyacinth contained antioxidizing agents. Out of these extracts, aqueous extract showed the most potent to DPPH is radical scavenging ability. The % inhibition of free radical scavenging activity of water hyacinth was shown in Table 1 and Figure 8.

Table 1.DPPH (2,2 diphenyl 1 picryl-hydrazyl) radical scavenging assay of different extracts of *Eichhornia crassipes*

Serial N0.	Control (abs)	Concentration (µL)	Sample (µL)	%Inhibition of free radical DPPH=(1-sample/control)x100%
1.	1.077	40	DW- 0.173 0.202 0.231	84 81 79
2.	1.091	40	EtOH- 0.452 0.797 0.778	59 27 29
3.	1.068	40	MEOH- 0.524 0.317 0.146	51 71 87

Table 2.Antidiabetes activity of different extracts of *Eichhornia crassipes*

Serial N0.	Control (abs)	Concentration (µL)	Sample (µL)	%Inhibition of free radical DPPH=(1-sample/control)x100%
1.	1.030	100	DW- 1.096 0.513 0.510	-6(No detect) 50 50
2.	0.794	100	EtOH- 0.649 0.247 0.244	18 69 69
3.	1.078	100	MEOH-0.442 0.454 0.443	59 58 59

Table 3.AchE activity of different extracts of *Eichhornia crassipes*

Serial N0.	Control (abs)	Concentration (µL)	Sample (µL)	%Inhibition of free radical DPPH=(1-sample/control)x100%
1.	2.571	150	DW- 1.945 1.940 2.081	24 25 19
2.	2.767	150	EtOH- 1.478 1.578 1.458	47 43 47
3.	2.960	150	MEOH-1.860 1.931 1.860	37 35 37

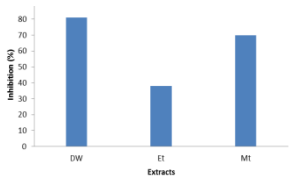


Figure 8. DPPH radical scavenging assay of different extracts of *Eichhornia crassipes*

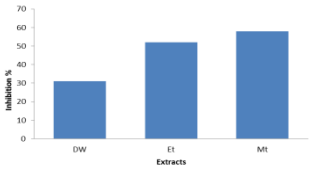


Figure 9. Antidiabetes activity of different extracts of *Eichhornia crassipes*

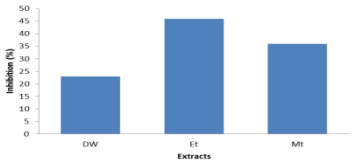


Figure 10.. AchE activity of different extracts of *Eichhornia crassipes*

2.Antidiabetic activity

There was inhibitory activity against α-glucosidase enzyme. The aqueous extract, the ethanol extract and methanol extract of water hyacinth show good inhibitory activity. The methanol extract of water hyacinths more inhibitory percentage than the other extracts. A comparative α- glucosidase percentage inhibition is given in Table 2, Figure 9.

3. AchE activity

The aqueous extract, ethanol extract and methanol extract of water hyacinth show inhibitory activity. The ethanol extract of water hyacinth shows more inhibitory activity than the other extracts. The percentage inhibition activity of AchE of water hyacinth are given in Table 3, Figure 10.

Table 4.Quantitative analysis of phenolic content of different extracts of *Eichhornia crassipes*

Serial N0.	Control (abs)	Concentration (µL)	Sample (µL)	Total phenolic content= S - 0.1523/0.5965
1.	700nm	100	DW- 0.978 0.994 1.035	1.3842 1.4111 1.4797
2.	700nm	100	EtOH- 0.134 0.464 0.717	-0.0273 0.5225 0.9467
3.	700nm	100	MEOH- 0.848 0.885 1.022	1.1663 1.2283 1.4580

Table 5.Quantitative analysis of flavonoid content of different extracts of *Eichhornia crassipes*

Serial N0.	Control (abs)	Concentration (µL)	Sample (µL)	Total flavonoid content= S - 0.0848 / 0.0002
1.	500nm	250	DW- 3.215 3.215 3.436	15651 15651 16756
2.	500nm	250	EtOH-1.012 1.038 1.084	4636 4766 4966
3.	500nm	250	MEOH- 1.444 1.471 1.459	6796 6931 6871

4. Total Phenolic Content

Maximum amount of total phenolic compound was observed in aqueous (distilled water) extract and minimum in ethanol extract of water hyacinth.Table - 4 and Figure 11 the isolated phenolic content in water hyacinth.

5. Total flavonoid Content

Maximum amount of total flavonoid compound was observed in aqueous (distilled water) extract and minimum in ethanol extract of water hyacinth.Table -5 and Figure 12 show the isolated flavonoid content in water hyacinth.

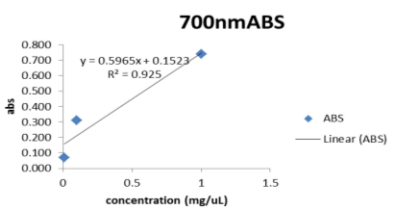
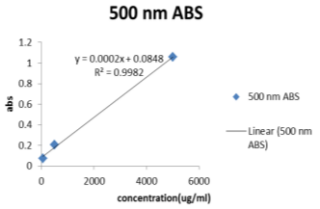


Figure 11. Standard of phenolic content

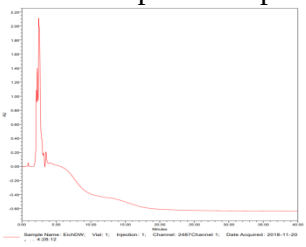


Figue 12. Standard of flavonoid content

HPLC detection

The presence of glutathione in the dry flower of *Eichhornia crassipes* has been detected by HPLC and estimated by glutathione assay. The HPLC of Glutathione (Std), aqueous extract, ethanol extract and methanol extract was recorded in the optimized chromatographic conditions. The composition of the mobile phase was optimized by using the concentration of two mobile phases. The composition 0.06% trifluoroacetic acid (TFA) in water (v/v) (A) and 100% acetonitrile (B) (50% A nd 50% B) was chosen for the study.

Glutathione showed two peaks at retention time (Rt) 0.18 and 5.52 min. The aqueous extract showed three peaks at Rt 0.18, 2.47 and 3.50 min.(Figure 13) The area of the peak at Rt 2.47 min was found to increase where as the other peaks showed a decrease. In comparison with the standard, the peak at Rt 2.47 min was assigned to glutathione. The ethanol extract showed four peaks at Rt 2.51, 4.11 and 5.01and 5.52 min(Figure 14). The area of the peak at Rt 2.51 min was found to increase where as the other peaks showed a decrease. In comparison with the standard, the peak at Rt 2.51 min was assigned to glutathione. The methanol extract showed four peaks at Rt 2.54, 3.35 and 4.97and 5.49 min.(Figure 15). The area of the peak at Rt 2.54 min was found to increase where as the other peaks showed a decrease. In comparison with the standard, the peak at Rt 2.54 min was assigned to glutathione. Trifluoacetic acid might have been lost to the head space above the eluent and would cause a progressive change in retention time during analysis. Estimation of reduced glutathione by the method of Moron *et al* (1979) indicated the optical density of the extract at 412 nm to 0,17 and 0,15. The results of the study reveal 1g of water hyacinth flowers to contain 0.1 mmole of reduced glutathione. Glutathione play a significant role in maintaining the protein –SH groups in the reduced state and removing toxic peroxides formed in metabolism. This may provide an opportunity for the plant to be used in pharmacological and cosmecutical industry since glutathione has been reported to possess strong antioxidant and antiageing.



Fiure 13. HPLC of the Aqueous extract of *Eichhornia crassipes*

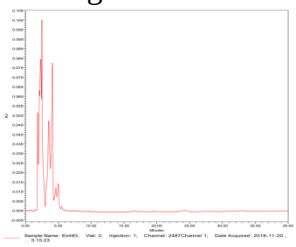


Figure 14. HPLC of Ethanol extract of *Eichhornia crassipes*

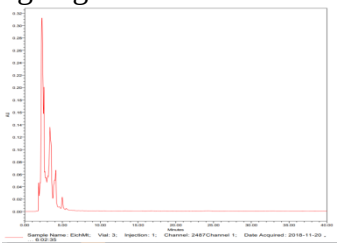


Figure15. HPLC of Methanol extract of *Eichhornia crassipes*

Conclusion

Aqueous, ethanol and methanol extracts of water hyacinth (*Eichhornia crassipes*) were investigated for anti-free radical activity by DPPH assay, antidiabetes and achE activity using UV. Total phenolic contents and flavonoid contents of flower extract of water hyacinth were determined. Moreover, HPLC analysis of aqueous, ethanol, methanol extracts of water hyacinth flower showed the plant to contain very bioactive compound, glutathione. This study tends to lead in the usefulness of the plant pharmaceutical and cosmetic industry. Further work involving the isolation of glutathione from water hyacinth may enhance the usefulness of the plant as it is available in plenty throughout the world.

Acknowledgements

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