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Antioxidant and Free Radical Scavenging Activity in Roots of *Hygrophila schulli* (Buch.-Ham.) M.R.Almeida & S.M. Almeida

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***Abstract*-** Nowadays herbal medicines form an important part of health care system, due to lots of side effects and complications in the modern medicine. The novel compounds synthesized in plants as secondary metabolites are the potential source of therapeutical uses. Plants produced significant amount of novel compounds to prevent the oxidative stress related diseases and can be used as antioxidant. One such attempt had been made in the present study in investigating the roots of *Hygrophila schulli* member of acanthaceae family for an antioxidant activity. The roots of *H.schulli* were extracted using petroleum ether as a solvent. Various assays such as DPPH (1,1-diphenyl 2-picryihydrazyl) free radical, ABTS radical cation, Phosphomolybdeunm reduction assay and ferric reducing power activity were checked for the antioxidant ability and free radical scavenging activity. In all these assays the root extract of *H.schulli* showed maximum reducing activity of free radical scavenging activity. The IC 50 value of the root extract in each assay was compared with the standard value of ascorbic acid and found *Hygrophila schulli* has a strong antioxidant and free Radical Scavenging Activity.

***Keywords*:** *Hygrophila schulli*, antioxidant, Free radical, DPPH, ABTS, Phosphomolybdeunm, Fe3+

I. INTRODUCTION

Hygrophila schulli (Buch.-Ham.) M.R.Almeida & S.M. Almeida an important medicinal plant belonging to the family Acanthaceae is distributed throughout India. *H. auriculata* (K. Schum) Heine and *Astercantha longifolia* (L.) Nees are the Synonyms of *Hygrophila schulli* [1]. This plant grows in water shed areas, like agriculture fields, tanks and ditches. This plant has been widely used in traditional medicine which date back to period of Ayurveda, Sidha, Unani etc. In ayurveda it is mentioned as Ikshura, Ikshugandha, and Kokilaaksha [2]. In Unani it is mentioned a Talmakhana and in Sidha it is mentioned as Neermulli [2]. In phytochemical analysis it is found that the roots of *H. schulli* contain Lupeol as major phytocompound, which is active compound in treating various diseases [3, 4, 5].

The World Health Organization (WHO) has estimated that 80% of the human population still relies on herbal medicine for their health care and for their daily use [6]. The active novel compounds present in the plants are responsible for the therapeutic property [7]. Nowadays the effects of oxidative stress on human health is a serious issue, during stress human body produce more reactive oxygen species (ROS) which leads to cell damage and health problems such as liver damage, cardiovascular diseases, inflammatory diseases neurodegenerative diseases, cancers etc[8, 9, 10]. In treatment of these diseases, antioxidant therapy has gained immense importance [11]. However the use of synthetic antioxidant lead to adverse effect, for this reason the interest in use of Plant as natural antioxidant has increased [12, 13, 14]. Medicinal Plants are the potential antioxidants which prevent oxidative damage by free radical and ROS.

This article deals with the research on antioxidant activity of the roots of *H. schulli*. The medicinal plant selected for the present study and the importance of antioxidant is briefly mentioned in Introduction Section of this article followed by the objective of the study. The plant collection, plant part extract preparation procedure using suitable solvent and various methodology used in investigating roots of *H. schulli* for antioxidant activity has clearly mentioned in Methods and Methodology Section. The results observed in various investigation is neatly tabulated and for clear understanding of the results it is represented with Graphs. The results are thoroughly discussed by comparing with previous works in Results and Discussion Section. Finally the article is concluded with recommendation in Conclusion Section.

II. MATERIALS AND METHODS

2.1 The plant collection

The fresh plant *H. schulli* were collected from the periphery of paddy filed in Alappatti village, Krishnagiri district, Tamil Nadu, India. Authentic identification of the plant was done by Botanical Survey of India, TNAU, South zone Coimbatore, Tamil Nadu.

2.2. The plant extracts preparation

The healthy plant roots of *H. schulli* were collected and sterilized by standard surface sterilization method. The roots were separated and dried in shade at room temperature till it was completely dried and were ground in to coarse powder. The root extract was prepared using petroleum ether as a solvent in soxhlation instruments. The obtained extract was vacuum dried and the concentrated extract was stored at 4^{o}C in an airtight container for further processing [2].

2.3. ANTIOXIDANT ACTIVITY

2.3.1. DPPH Radical scavenging activity

The antioxidant activity of *H. schulli* roots were measured on the basis of the scavenging activity of the stable 1,1-diphenyl 2-picryihydrazyl (DPPH) free radical with the slight modification of standard procedure [15]. 1 ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations *H. schulli* root extract in petroleum ether (10-120 µg/ml). The mixture was then incubated in dark for about 30 minutes. Mixer of 1ml methanol and 1 ml DPPH solution was used as the control and Ascorbic acid was used as reference standard. The variation in absorbance was measured using UV-Vis spectrophotometer at 517nm. The percentage of inhibition was calculated the following formula.

2.3.2. ABTS $^{\bullet+}$ Radical scavenging activity

The antioxidant capacity was determined in terms of the ABTS $^{\bullet+}$ radical cation decolorization assay. ABTS$^{\bullet+}$ radical cation was produced by the reaction between 7mM of ABTS and 2.45 mM potassium persulfate in water and stored in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 734nm of 0.70±0.02. To various concentrations (10-60 µg/ml) of petroleum ether extract in root of *H. schulli*, with 500 µL of diluted ABTS$^{\bullet+}$ solution was added and incubated at room temperature 10 min. the absorbance was measured at 734 nm. Ascorbic acid was used as reference standard. The ABTS$^{\bullet+}$ radical cation scavenging activity was expressed as % of ABTS$^{\bullet+}$ radical cation inhibition and calculated using the standard formula [16].

2.3.3. Phosphomolybdenum reduction assay

The antioxidant capacity of *H. schulli* root in petroleum ether extract was assessed by phosphomolybdenum reduction method [17]. The root extract of *H. schulli* in different concentration (20-120 µg/mL) was combined with 1ml of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95ºC for 90 min. After, the mixture was cooled to room temperature and the absorbance of the colored complex was measured at 695 nm. The percentage of reduction was calculated using the standard formula

2.3.4. Ferric (Fe $^{3+}$) reducing power assay

The Fe^{3+} reducing power of *H. schulli* root in petroleum ether extract was determined by using potassium ferricyanide assay method with slight modification [18]. One mL of root extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v) solution. The mixtures were then incubated at 50ºC for 30 min in a water bath. Five hundred µL of trichloroacetic acid (10% w/v) was added and shaken well followed by 100 µL of freshly prepared $FeCl_3$ (0.1% w/v) solution was added and shaken well. The absorbance was measured at 700 nm and the percentage of reduction was calculated.

III. RESULTS AND DISCUSSION

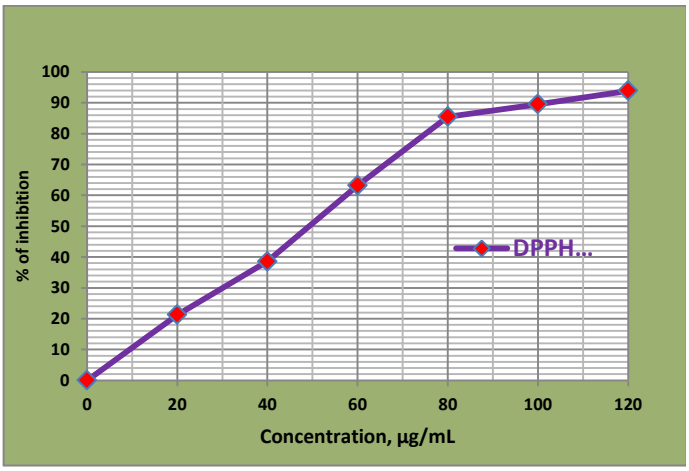
3.1. DPPH Radical scavenging activity

The roots of *H.schulli* in petroleum ether extract was assessed using 1, 1- diphenyl 2-picryihydrazyl (DPPH) for scavenging free radicals at 517 nm. DPPH is a nitrogen centered free radical, color of which changes from violet to yellow on reduction, reducing capacity is increased with increasing the concentration of the extract. The maximum percentage of DPPH radical scavenging activity was 93.91±6.57 at 120 µg/mL concentration as shown in **Table 3.1** and **Graph 3.1**. It was compared with the standard ascorbic acid 6.42 (µg/mL) and IC_{50} of DPPH radical scavenging activity was 51.84 (µg/mL) Concentration. The scavenging ability of petroleum ether extract of roots of *H.schulli* may be due to the bio compositions of phenolic acids and flavonoid [19].

The DPPH activity of the root extract of *H.schulli* was compared with the earlier work in seed extract of *H.schulli* and found similar pattern of antioxidant ability [20].

Table 3.1. DPPH Radical scavenging activity

S. No.	Concentration (µg/mL)	% of inhibition DPPH at 517 nm
1	20	21.28±1.48
2	40	38.58±2.69
3	60	63.18±4.42
4	80	85.50±5.98
5	100	89.52±6.26
6	120	93.91±6.57



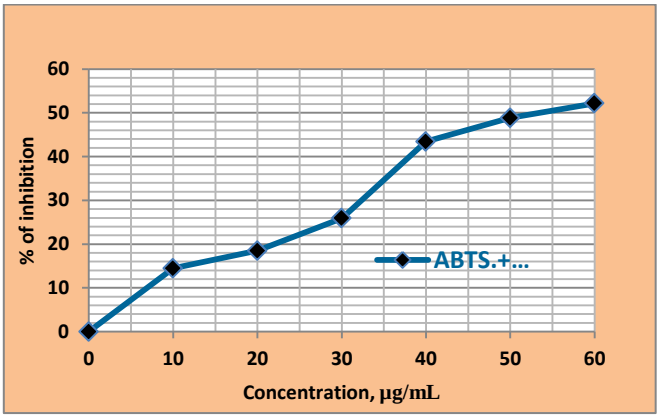
Graph 3.1. DPPH Radical scavenging activity

3.2. ABTS $^{\bullet+}$ Radical scavenging activity

Antioxidant activity was assessed for ABTS $^{\bullet+}$ radical cation scavenging activity, a blue chromophore was produced in the reaction when ABTS, potassium persulfate and root extract were subjected together, the released cation radical gets reduced and the remaining free radical cation concentration was then quantified [20]. The maximum percentage of ABTS$^{\bullet+}$ free radical scavenging activity was determined as 52.18±3.65 % at 60 µg/mL concentration, as shown in **Table 3.2** and **Graph 3.2**. It was compared with the standard ascorbic acid 5.83 (µg/mL) and IC_{50} of ABTS $^{\bullet+}$ radical cation scavenging activity was 51.21 (µg/mL) concentration.

Table 3.2. ABTS $^{\bullet+}$ Radical scavenging activity

S. No.	Concentration (µg/mL)	% of inhibition ABTS$^{\cdot+}$ at 734 nm
1	10	14.47±1.01
2	20	18.51±1.29
3	30	25.92±1.81
4	40	43.43±3.04
5	50	48.82±3.41
6	60	52.18±3.65



Graph 3.2. ABTS $^{\bullet+}$ Radical scavenging activity

3.3. Phosphomolybdenum reduction assay

The total antioxidant activity of root extract of *H. schulli* was measured for phosphomolybdenum reduction assay at 695 nm, where Mo (VI) is reduced to green phosphate/Mo (V) complex at acidic pH [21]. The maximum absorbance was 86.23±6.03 at 120 µg/mL concentrations (**Table 3.3** and **Graph 3.3**), which was compared with standard 3.09 (µg/mL) ascorbic acid and the IC_{50} of phosphomolybdenum reduction activity was 107.41 (µg/mL) concentrations.

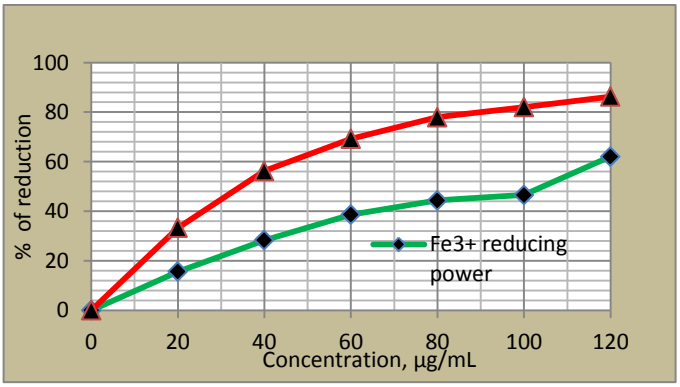
3.4. Ferric (Fe $^{3+}$) reducing power assay

Ferric (Fe $^{3+}$) reducing power activity was measured in root extract of *H. schulli* at 700nm. In the assay Fe^{3+} was reduced to Fe^{2+} as the concentration of the extract increased, there was increase reducing activity [21]. The maximum reducing activity and free radical scavenger was 62.07±4.34 at 120 (µg/mL) concentrations (**Table 3.3** and **Graph 3.3**). The reducing ability increases with the increase in the extract concentration. It was compared with standard ascorbic acid

4.50 (µg/mL) concentration and IC $_{50}$ Ferric (Fe^{3+}) reduction power activity 35.56 (µg/mL) concentrations.

Table 3.3. Phosphomolybdenum reduction and ferric (Fe^{3+}) reducing power assay

S. No.	Concentration (µg/mL)	% of reduction	
		Phosphomolybdenum reduction at 695 nm	Fe^{3+} reducing power at 700 nm
1	20	33.33±2.33	15.64±1.09
2	40	56.25±3.93	28.32±1.98
3	60	69.23±4.48	38.61±2.70
4	80	77.95±5.45	44.39±3.10
5	100	82.05±5.74	46.55±3.25
6	120	86.23±6.03	62.07±4.34



Graph 3.3. Phosphomolybdenum reduction and ferric (Fe^{3+}) reducing power assay

VI. CONCLUSION

In this study the roots of *H.schulli* was subjected to various investigations such as DPPH Radical scavenging activity, ABTS $^{\bullet+}$ Radical scavenging activity, Phosphomolybdenum reduction assay and Ferric (Fe $^{3+}$) reducing power assay to study the antioxidant ability and free radical scavenging activity. It was clearly observed in each investigation the maximum percentage of free radical scavenging activity was determined and strong reducing capacity-antioxidant was noted. It was also noted the reducing capacity increased with the concentration of the extract. The results were compared with the standard ascorbic acid and IC_{50} value of free radical scavenging activity was determined. The result showed increased (reducing capacity) antioxidant activity and is recommended that the root of *H.schulli* is good source for antioxidant and radical scavenging process.

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