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In vitro Evaluation of Antioxidant properties of *Hodgsonia heteroclita* (Cucurbitaceae) fruit

Silu Basumatary[1*], Ajit Kumar Das[1], Raaman Nanjian[2], Gauri Dutt Sharma[3]

[1]Ethnobotany and Conservation of Medicinal Plants Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar-78801, India. [2]Laboratory of Natural Products, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600025 (Tamil Nadu) India. [3]Bilaspur University, Bilaspur -495009(Chattishgarh) India.

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ABSTRACT

Hodgsonia heteroclita fruits have been used as traditional drug for antidiabetic in Western Assam, India. The study aimed to investigate antioxidant activity of methanolic fruit extract of *H. heteroclita*. The antioxidant activity of fruit was determined by employing different *In vitro* assays such as DPPH•, ABTS•+ radical scavenging capacities and reducing power assay-phosphomolybdenum. The results of the present study revealed that the methanol fruit extract exhibited strong scavenging effect on DPPH (2,2-diphenyl-1picrylhydrazyl) with IC50 =20.98±0.17 than ABTS [2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonate) with IC50 =28.58±0.42. The reducing power phosphomolybdenum assay showed inhibition with ascorbic acid as standard. Fruit extract exhibit free radical scavenging and reducing properties which might constitute an important source of natural antioxidants. Thus the study indicates that this plant has a potential to be used as an antioxidant and also can be used to prevent diseases directly linked to oxidative stress.

INTRODUCTION

Traditional knowledge of using medicinal plants in North East India has been transferred from one generation to other through ages. Plants are the good resources for natural antioxidants therefore natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. Oxidative stress has been linked to serious diseases like cancer, heart diseases, stroke, aging, diabetes, arthritis etc. Antioxidants are substances that are able to counter free radical and they may help to suppress the imbalance that occur during oxidative stress. Natural antioxidants are widely used because they are safe and cause the adverse reactions (Boik, 1996). Antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation (Dekkers *et al*., 1996) and it is one of the most essential ingredients of today's therapy since they reduce in vivo oxidative damages. DPPH radical scavenging method is widely used to investigate the total antioxidant activities in plants. Free radicals together with other derivatives of oxygen are inevitable by products of biological redox reactions. The potential toxicity of synthetic antioxidants (hydroxyanisole-BHA, butylated hydroxytoluene-BHT, tertiary butylhydroquinone, esters of 3, 4, 5- trihydroxybenzoic acid etc.) has aroused an interest and scientists have focused on isolation and characterization of antioxidants from natural sources such as herbs, spices, seeds, cereals, fruits and vegetables by extraction, fractionation and purification (Dillard and German, 2000; Wang & Linn, 2000).

Hogdsonia heteroclita belongs to the family Cucurbitaceae, locally known as "Hagrani jwgwnar" which is bitter in taste and used in traditional system of medicine for curing diabetes in Kokrajhar district of Western Assam (Swargiary *et al*., 2013; Basumatary *et al*., 2015). Although the roots and the fruits of plant belonging to Cucurbitaceae species are very bitter, they have been used as folk medicines in some countries because of their wide spectrum of pharmacological activities. In Northeast India, the fruit bulb *H. heteroclita* is applied to bacterial infections in the feet (Changkija, 1999), intestinal worms (Semwal *et al*., 2014).

Hence in this study the *Hodgsonia heteroclita* species was selected to analyze its therapeutic importance with respect to antioxidant effects.

* *Corresponding Author*
Silu Basumatary, Ethnobotany and Conservation of Medicinal Plants Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar-78801, India. Email: silubasumatary@gmail.com

MATERIALS AND METHODS

Plant material

The fruits of *Hodgsonia heteroclita* were collected from Kokrajhar district of Western Assam. The fruits were harvested in the month of March-April. The fruit part of the plant was identified and authenticated by the Botanical Survey of India, Eastern Circle Shillong.

PREPARATION OF PLANT EXTRACT

The fruits of the plant were thoroughly washed and the fruit pulp is cut into pieces, shade dried and air dried at room temperature. Dried material was ground to a fine powder and stored at ambient temperature till use. 10 gms of the powdered plant material was extracted with 100 ml of methanol for 72 hours and shaken in Rotary shaker. The extract was then filtered and concentrated in Rotary Vapor to get the methanol fruit extract.

CHEMICALS AND REAGENTS REQUIRED

DPPH (2, 2- Diphenyl-1- picryl hydrazyl) and ABTS [2,2-Azino-bis (3-ethylbenzothiazoline-6 sulfonate)] were purchased from Sigma. Quercetin and Ascorbic acid were purchased from Alfa Aesar, Mumbai, India. Disodium phosphate ($Na_2HPO_4.2H_2O$), Ammonium molybdate $(NH_4)_6$ $Mo7O_{24}$ and Hydrochloric acid (H_2SO_4), Methanol were purchased from Qualigen India Limited.

Determination of DPPH free radical scavenging activity:

Radical scavenging activity of *H. heteroclita* methanolic fruit extract was evaluated by using the method (Brand Williams *et al*., 1995; Molyneux, 2004; Li *et al*., 2009). A stock solution of DPPH at a concentration of 0.1mM was prepared. To the test tubes 1mL of this solution was added to 1mL of extract solution of methanol at different concentrations (5- 100 µg/mL). Control was prepared with equal volume of methanol and DPPH without adding sample.

The mixture taken in the test tubes were shaken vigorously and left to stand in the dark for incubation for 30 min at room temperature. Control was prepared with equal volume of methanol and DPPH without adding sample.

Then absorbance was measured at 517 nm using "Beckman DU-40" spectrophotometer against methanol as blank. Quercetin at various concentrations was used as the standard reference. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The experiments were carried out in triplicates. The radical scavenging activity was calculated by the following formula;

$$\% \text{ of scavenging} = \text{Control- Sample/Control} \times 100$$

The results were expressed as mean value ± standard deviations. IC_{50} value represents the levels at which 50 % of the radicals are scavenged by test samples.

Determination of Total antioxidant capacity by Phosphomolybdenum reduction assay

Procedure

The total antioxidant capacity of the methanol extract was evaluated by phosphomolybdenum assay described by (Preito *et al*., 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate – Mo (V) complex at acid pH. Extract with different concentrations (10- 100 µg/mL) were taken in test tubes and combined with 1mL of reagent solution of (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the solution reaction were capped and incubated in a boiling water bath at 90 °C for 90 minutes. After cooling to room temperature, the absorbance of solution was measured at 695 nm against water as blank by a spectrophotometer. For reference the appropriate solutions of ascorbic acid have been used. All tests were run in triplicates.

Determination of ABTS free radical scavenging

$ABTS^{\cdot+}$ free radical was determined according to a method reported by (Pari and Suresh, 2008). ABTS was generated by the interaction of 5.0 ml ABTS solution (1.8mM) mixed with 1.25mL potassium persulphate (2.0 mM) and kept in dark at room temperature for 2 hours, then diluted five times with phosphate buffer pH 7.0 (0.02mM). Then 0.4 mL of various concentrations of samples in methanol were taken and mixed with 3.6 mL of ABTS solution and kept in the dark for 10 minutes. The absorbance was measured at 734nm using "Beckman DU-40" spectrophotometer. Ascorbic acid at various concentrations was used as the standard reference. The control was prepared without the extract and the experiment was carried out on triplicates. ABTS scavenging effect was calculated as percentage of ABTS scavenging using the following equation.

$$\% \text{ scavenging} = A_{ABTS} - A_S \times 100 / A_{ABTS}$$

STATISTICAL ANALYSIS

All evaluations of antioxidant activity were performed in triplicates. Data were expressed as means ± standard deviation.

RESULTS AND DISCUSSIONS

In the present study, three commonly used antioxidant evaluation methods such as DPPH, ABTS radical scavenging activity and phosphomolybdenum reducing power assays were used to determine the antioxidant potential of *Hodgsonia heteroclita* fruit extract.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity. In comparison to standard quercetin the percentage of inhibition of DPPH radical scavenging activity of the methanol fruit extract ranged from 34.37µg/mL to 96.48 at 100µg/mL while quercetin exhibited stronger inhibition than methanol extract which ranged from 13.52µg/ml to 72.94µg/ml at different concentration shown in figure (1 & 2).

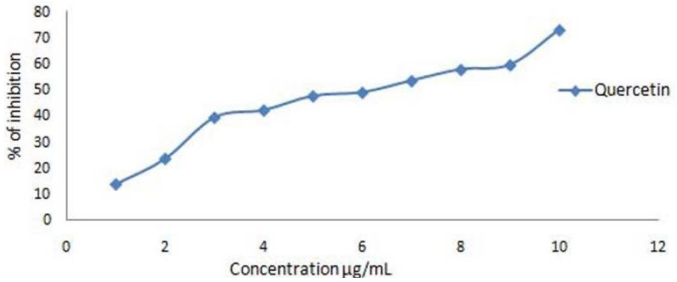

Fig. 1: DPPH Scavenging Assay with Quercetin as Standard.

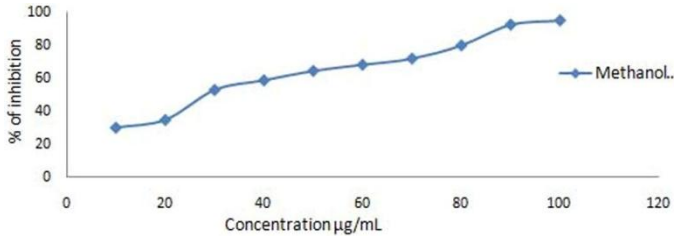

Fig. 2: DPPH Scavengin Assay of Methanol extract.

ABTS radical inhibition ranged from 15.38µg/mL to 94.82µg/mL at 100µg/mL concentration for methanol extract and ascorbic acid as standard showed inhibition from 15.38µ to 73.9µg/mLg/ml at different concentration shown in figure (3 & 4). The activity of inhibition increased with the increasing concentration. The phosphomolybdenum reduction assay increases with increases in concentration of methanol extract of fruit of *Hodgsonia heteroclita* as shown in table 1. The reduction power assay showed greater reduction capacity compared with the standard ascorbic acid.

Table 1: Phosphomolybdenum Assay of the Methanol extract compared with standard as Ascorbic acid.

Concentration µg/mL	Phosphomolybdenum assay of methanol extract (Absorbance at 695nm)	Standard as Ascorbic acid (Absorbance at 695 nm)
10	0.012±0.001	0.003±0.001
20	0.015±0.001	0.004±0.001
30	0.027±0.001	0.007±0.001
40	0.035±0.001	0.009±0.001
50	0.047±0.001	0.015±0.001
60	0.051±0.001	0.020±0.001
70	0.058±0.002	0.028±0.001
80	0.062±0.002	0.035±0.001
90	0.067±0.002	0.042±0.001
100	0.070±0.002	0.051±0.002

IC_{50} indicate the potency of scavenging activity. Methanol fruit extract showed highest $DPPH^{+}$ radical scavenging activity (IC_{50} = 20.98) than $ABTS^{+}$ with IC_{50} = 28.58µg/mL as shown in table 2.

Table 2: IC_{50} value of DPPH and ABTS assay of methanolic fruit extract

Plant part	Plant Extract	IC 50 µg/mL	
		DPPH	ABTS
Fruit	Methanol	20.98±0.17	28.58±0.42

Oxidative stress is now recognized to be associated with more than 100 diseases as well as with normal aging process (Ghansanfari *et al*., 2006). Antioxidants are intimately involved in the prevention of cellular damage. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecues (Halliwell *et al*., 1985; Halliwell, 1994) e.g. cerebrovascular disease, cancer, heart diseases, aging, arthritis, diabetes mellitus, stroke (Sharma & Clark, 1998).

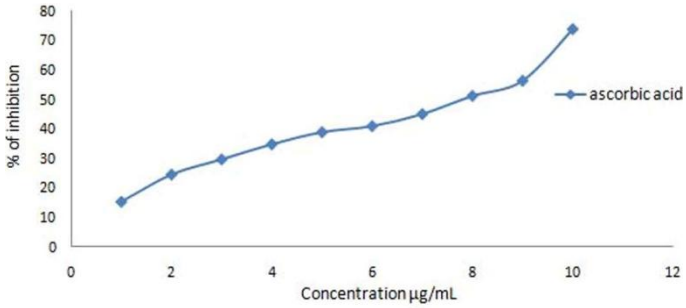

Fig. 3: ABTS Assay with Ascorbic acid as standard.

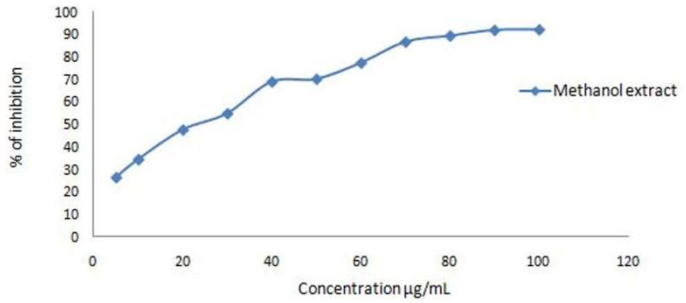

Fig. 4: ABTS Assay of Methanol extract.

Excess production of free radicals or decrease in antioxidant level leads to oxidative stress. The principle of antioxidant activity is their interaction to produce oxidative free radicals. Radical scavenging antioxidants are particularly important in antioxidative-defence in protecting cells from the injury of free radical (Youwei *et al*., 2008).

The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH (α, α- diphenyl-β-picrylhydrazyl) is converted into α-α-diphenyl-β-picrylhydrazine with colour change. DPPH radical is reduced to the corresponding hydrazine, a colour change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. DPPH• and ABTS•+ scavenging activities involve hydrogen atoms transfer and electrons transfer. The results of the present investigation explains that the methanol extract of fruit of *H. heteroclita* may contain enormous amount of hydrogen donor molecules which may reduce the production of radicals and the decolorization in the DPPH• and ABTS•+ assays. The presence of specific chemical compounds in the extract (Basumatary *et al*., 2015) may inhibit the potassium persulphate activity and hence reduced the production of ABTS•+. The phosphomolybdenum assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green complex at acid pH..

CONCLUSION

An *In vitro* of the present study concludes that the methanolic fruit extract of *Hodgsonia heteroclita* posses significant antioxidant activity.

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