Antioxidant activity of *Stevia rebaudiana* Bert. Leaves from Bangladesh

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Abstract

The *in vitro* antioxidant potential of different extractives of *Stevia rebaudiana* Bert. was evaluated in the present study. Four complementary test systems; namely DPPH free radical scavenging, reducing power, total phenolic and total flavonoids concentrations were used for this study. IC_{50} values of 80% ethanol extract (at room temp.) and its 1-butanol and water soluble fractions were found to be 8.02±0.874, 23.60±0.763 and 43.81± 0.459 µg/mL, respectively and that for methanol and water hot extracts were observed as 44.61±0.821 and 23.70±0.861 µg/mL, respectively. Ascorbic acid, the standard compound exhibited the IC_{50} value 4.21±0.861. In reducing power test the maximum absorbance for 80% ethanol extract and its, n-hexane, dichloromethane, 1-butanol and water soluble fractions were found to be upto 1.0717±0.0017, 0.5684±0.0013, 0.8191±0.0017, 0.9819±0.0014 and 1.5552±0.0015, respectively and that for hot extracts, n-hexane, dichloromethane, methanol and water were upto 0.5894±0.0014, 0.9498±,0.0015, 0.9086±0.0018 and 0.9972±0.0029, respectively compared to the absorbance of ascorbic acid as standard (1.3741±0.0031). Total phenolic concentrations in 80% ethanol extract (at room temp.) and its different fractions were ranged from 25.36±0.34 - 65.21±0.97 and that in different hot extracts were observed in the range 15.33 ±0.78,36.95±0.09 mg gm^{-1} gallic acid equivalent, respectively. The total flavonoids concentration of 80% ethanol extract and its fractions were ranged from 34.26±0.79 -125.64±1.07 and the range for hot extracts was 23.56±0.89 - 76.94±0.35 mg gm^{-1} quercetin equivalent per gm of dry extract, respectively. The results revealed that 80% ethanol extract exhibited most significant antioxidant activity followed by its water and 1-butanol extracts and hot methanol and water extracts. This clearly indicates that *Stevia rebaudiana* leaves from Bangladesh has a significant potential to use as a natural antioxidant.

Key words: *Stevia rebaudiana* Bert. Antioxidant activity, DPPH free radical scavenging, Reducing power, Total phenolic and Total flavonoids

Introduction

Oxidation is essential to human beings to fuel biological processes for the production of energy. During metabolism, molecular oxygen is reduced to water; yet the stepwise transfer of electrons generates free reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet}$-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). Other radicals such as alkyl ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$), and peroxyl ($ROO^{\bullet}$) radicals may also be produced endogenously (Simic *et al.*, 1989). The uncontrolled production of oxygen derived free radicals is involved in onset of many diseases such as aging, immunodeficiencies, neurologic disorders, inflammation, arthritis, ischemia, arteriosclerosis, coronary heart disease, stroke, diabetes mellitus, Parkinson's disease, Alzheimer's disease and certain cancers (Sies, 1991; Gutteridge, 1993; Kehrer, 1993; Aruoma, 1994; Cook *et al.*, 1996; Scandalios, 1997; Kumpulainen *et al.*, 1999; Halliwell *et al*, 1999; Parejo *et al.*, 2002; Hou *et al.*, 2003;). Allmost all organisms are well protected against free radical damage by enzymes such superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau *et al.*, 2002). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging,

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deterioration of physiological functions may occur in diseases and accelerated aging. There is a balance between generation of ROS and their removal by the antioxidant system in organisms. Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well known abilities to scavenge free radicals i.e. antioxidant power (Hou *et al.*, 2003; Galvez *et al.*, 2005; Kukic *et al.*, 2006).

Stevia rebaudiana Bert. (Family: Asteraceae) widely known as "Sweet-Leaf" and simply "stevia", is an herbaceous perennial shrub. It is widely cultivated and used in different items of food in South America, Central America, Mexico and East Asia. Now a days, stevia is cultivated in many countries of South East Asia including Bangladesh. Over 100 Phytochemicals have been discovered in stevia so far. The constituents responsible for stevia's sweetness were documented in 1931, when eight novel plant chemicals called glycosides were discovered. Of these eight glycosides, one called *stevioside* is considered the sweetest one and it has been tested to be approximately 300 times sweeter than sugar (Bridel *et al.*, 1931; Adduci, *et al.*, 1987; Liu *et al.*, 1995). Stevioside, comprising 6-18% of the stevia leaf, is also the most prevalent glycoside in the leaf. Stevioside acts as an antihyperglycemic and an effective antihypertensive substance (Gregersen *et al.*, 2004; Chang *et al.*, 2005; Liu *et. al.*, 2003; Hsu *et. al.*, 2002; Chan *et al.*, 1998). Other sweet constituents of stevia include steviobioside, rebausiosides A-E, and dulcoside A (Kinghorn *et al.*, 1984). The non sweet constituents identified in *S. rebaudaiana* leaves are labdane diterpenes, triterpenes, sterols, flavonoids, volatile oil constituents, pigments and inorganic matters (Kinghorn 1992). The ethanolic extract of *Stevia rebaudiana* from India has been reported to have antioxidant properties (Shukla *et al.*, 2009).

The bioactivity of any plant products greatly varies with the change of geographical conditions, such as soil, water cultivation process etc. So far, the antioxidant capacity of *Stevia rebaudiana* cultivated in Bangladesh has not been evaluated. The present paper is reporting the antioxidant potential of the different extractives of *Stevia rebaudiana* from Bangladesh.

Materials and Methods

Collection of plant materials

Two kg *Stevia rebaudiana* leaves powder purchased from BRAC Nursery centre, Gazipur during 2008.

Analytical materials

Analytical or laboratory grade (BDH, E Merck) solvents Ethanol, n-hexane, dichloromethane, methanol, were used for the extraction and fractionation. All evaporations were carried out under reduced pressure using a vacuum rotary evaporator (Buchi, Switzerland) at water bath temperature not exceeding 40°C. Freeze-drying of aqueous extracts and fractions were carried out with Varian 801 model LY-3-TT freeze-dryer. 1,1-Diphenyl-2-picryl hydrazyl (DPPH), L- ascorbic acid, Butylated hydroxyanisole, Gallic acid , Quarcetin, Folin-ciocalteu phenol reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), Phosphate buffer (pH 6.6), potassium ferricyanide [$K_3Fe(CN)_6$], $FeCl_3$, Na_2CO_3, aluminum chloride and potassium acetate were of analytical grade. A double beam Analykjena UV/Visible spectrophotometer (Specord 205, Germany) was used to record the absobance of the samples for antioxidant activity tests.

Preparation of samples

i) Extraction and fractionation of *Stevia rebaudiana* leaves powder at room temp: Dried *Stevia rebaudiana* (750.0g) was extracted by shaking with 80% ethanol (1.0L) at room temperature (28-30°C) for 72 hours. The extract was filtered through Buchner funnel and the extraction of the residue was repeated twice following the same method. All the filtrate together was concentrated with rotary vacuum evaporator at bath temperature not exceeding 40°C and then freeze dried. Dry 80% ethanol extracts (SR_1) 75.52g was obtained.

Fifty gram (50 g) of ethanol extract (SR_1) was partitioned between n-hexane and water (1:1). The n-hexane soluble part was separated and the aqueous part was further partitioned until the color of the n-hexane soluble part is almost colorless. The water soluble part was then partitioned with dichloromethane and the same procedure was followed as partitioned with n-hexane. After separation of the dichloromethane soluble part the

aqueous part was further partitioned with 1-butanol as above. All the solvent fractions were concentrated separately and then freeze dried. The amount of different fractions obtained was, n-hexane soluble fraction (SR_2: 3.12g), dichloromethane soluble fraction (SR_3: 4.40g), 1-butanol soluble fraction (SR_4: 35.16g) and water soluble fraction (SR_6: 5.36g).

ii) Soxhlet extraction of *Stevia rebaudiana* leaf powder: The *S. rebaudiana* leaf powder was successively extracted with n-hexane, dichloromethane and methanol for 10 hours by using soxhlet apparatus. The residue was then extracted with hot distilled water for ten hours and filtered. All the solvent extracts were concentrated separately and then freeze dried. Four hot extracts n-hexane (SR_7: 2.10g), dichloromethane (SR_8: 0.66g), methanol (SR_9: 4.73 g) and water (SR_{10}: 2.32 g) obtained.

Test for antioxidant activity: Four complementary test systems; namely DPPH free radical scavenging, reducing power, total phenolic and total flavonoids concentrations were used during the present study

i) DPPH radical scavenging activity: DPPH, a stable nitrogen centered radical was used to assess the hydrogen donating ability of different solvent extracts and fractions of *S rebaudiana* leaves as it offers a convenient and accurate method because of the relatively short time required for analysis. For assessing the DPPH radical scavenging activity, the modified method described by (Gupta *et. al.,* 2003) was used. Stock solution (5mg/mL) of the different solvent extracts and fractions of *S. rebaudiana* were prepared in respective solvent systems. Serial dilutions of the stock solution were carried out to obtain concentrations of 1, 5, 10, 50 and 100µg/mL. In this assay, an equal amount of sample solution (2 mL) was added to an equal amount of 0.1 mM methanolic DPPH solution. The mixture was vortex for few minutes and allowed to stand in dark place at 25°C for 30min for reaction to occur. After 30min of incubation, the absorbance was read against a blank at 517nm. The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

I (%) = (Absorbance of the blank – Absorbance of the sample / Absorbance of the blank) x 100

Where blank is the control sample reaction (containing all reagents except the test compound). The concentration of sample required to scavenge 50% DPPH free radical (IC_{50} value) was calculated from the plot of percent inhibition against the concentration of the test samples. All the tests were carried out in triplicate and average of the absorbance was recorded for each time. Butylated hydroxyanisole (BHA) was used as positive control standard.

ii) Reducing power assay: The reducing power of different solvent extracts and fractions of *stevia rebaudiana* leaves was determined according to the method previously described by (Oyaizu *et.al.*, 1986). 1 mL of test samples of different concentrations (1, 5, 10, 50, 100µg/mL) was mixed with phosphate buffer (2.5 mL) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50°C for 20min. 2.5mL of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solution was separated and mixed with distilled water (2.5mL), $FeCl_3$ (0.5mL) and the absorbance was measured against a blank at 700nm. Increased absorbance of the reaction mixture indicated the increased reducing power. All the tests were carried out in triplicate and average absorption was noted for each time. Butylated hydroxyanisole (BHA) was used as positive control.

iii) Assay for total phenolic concentration: The total phenolic concentration of the extracts and fractions of *Stevia rebaudiana* leaf was determined by the modified Folin-Ciocaltu method (Wolfe *et al.*, 2003). Briefly, 0.5mL of each extract/fraction (1mg/ml) was mixed with 5ml Folin-Ciocaltu reagent (1:10v/v distilled water) and 4 ml (75g/L) of Sodium carbonate. The mixture was vortexed for 15 second and allowed to stand for 30min at 40°C for color development and the absorbance was read at 765nm. Total phenolic content was determined as mg of Gallic acid equivalent per gram using the equation, obtained from a standard Gallic acid calibration curve y=6.9104x -0.0937, R^2=0.9972.

iv) Assay for total flavonoids concentration: Aluminium chloride colorimetric method (Chang *et al.,* 2002) was used for determination of total

flavonoids concentration in the samples of *Stevia rebaudiana* leaf. Each extract and fraction (0.5 ml, 1:10 gml^{-1}) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. Total flavonoids content was determined as mg of Quercetin equivalent per gram using the equation obtained from a standard Quercetin calibration curve $y=4.7385x + 0.0355$; $R^2 = 0.9993$

Results and Discussion

The present study was carried out to evaluate the *in vitro* antioxidant potential of different organic and aqueous soluble materials of potential of *Stevia rebaudiana* Bert. (Leaves) from Bangladesh. Two methods were followed for the extraction of *S. rebaudiana* leaves, i) extraction and fractionations at room temperature and ii) a successive hot extraction by soxhlet apparatus. Four complementary test systems; namely DPPH free radical scavenging, reducing power, total phenolics and total flavonoids concentration determinations were used for this study. The results are presented in Tables 1-5 & Figures 1-4.

Table 1: DPPH free radical scavenging activity of 80% ethanol extract (at room temp) and its different solvent fractions of *Stevia rebaudiana* and ascorbic acid

Extracts/ fractions/Standard	% Inhibition at different concentration (µg/mL)					IC_{50} (µg/mL)
	1	5	10	50	100	
SR_1	2.70±0.547	33.05±0.712	60.86±1.012	93.38±0.953	95.59±0.714	8.02±0.874
SR_2	1.79±0.852	2.01±0.542	4.41±0.451	5.92±0.452	7.95±0.897	---
SR_3	2.87±0.921	5.08±0.421	17.09±0.426	21.46±0.621	41.67±0.486	---
SR_4	1.02±0.784	6.82±0.855	33.82±0.754	81.66±0.411	95.38±0.675	23.60±0.763
SR_5	1.33±0.459	5.67±0.978	21.69±0.714	55.24±0.532	62.81±0.721	43.81±0.459
AsA	10.88±0.431	57.97±0.461	95.25±0.881	96.27±1.085	96.32±0.379	4.21±0.861

The values are expressed as mean±SD (n=3). AsA: Ascorbic acid.

Table 2: DPPH free radical scavenging activity of different solvent extracts (hot) of *Stevia rebaudiana*

Extracts/ Standard	% Inhibition at different concentration(µg/mL)					IC_{50} (µg/mL)
	1	5	10	50	100	
SR_6	1.02±0.245	10.23±0.758	16.33±0.549	20.22±0.688	21.89±0.911	---
SR_7	3.14±0.187	13.4±0.986	17.17±0.975	24.93±0.759	26.47±0.784	---
SR_8	5.39±0.265	11.63±0.457	35.49±1.447	52.28±0.694	88.69±0.841	44.61±0.821
SR_9	4.03±0.724	12.41±0.949	30.23±0.686	88.08±0.757	88.43±0.549	23.7±0.711
AsA	10.88±0.843	57.97±0.359	95.25±0.489	96.27±0.843	96.32±0.743	4.21±0.924

The values are expressed as mean±SD (n=3). AsA: Ascorbic acid.

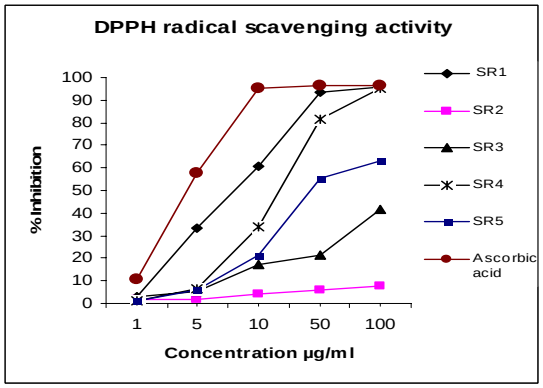

Figure 1: DPPH radical scavenging activity of 80% ethanol extract (room temp) and its different solvent fractions of *Stevia rebaudiana* and ascorbic acid. SR1: 80% ethanol extract; SR2: n-Hexane soluble fraction of SR1; SR3: Dichloromethane soluble fraction of SR1; SR4: 1-Butanol soluble fraction of SR1; SR5: Water soluble fraction of SR1.

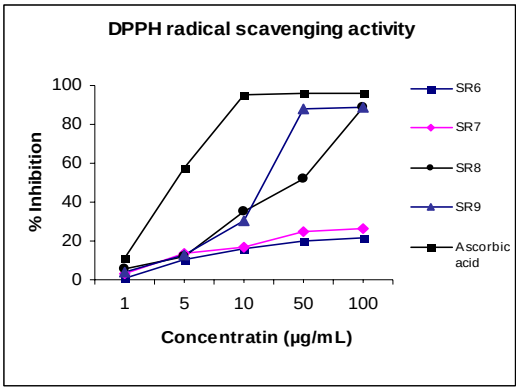

Figure 2: DPPH radical scavenging activity of different solvent extracts (hot) of *Stevia rebaudiana* and ascorbic acid. SR6: n-hexane extract; SR7: dichloromethane extract; SR8: methanol extract; SR9: water extract.

Table 3: Average absorbance at 700 nm of 80% ethanol extract (at room temp) and its different solvent fractions of *Stevia rebaudiana* and ascorbic acid for determination of reducing power

Extracts /Standard	Average absorbance at 700 nm at different concentration (µg/mL)				
	1	5	10	50	100
SR_1	0.4843±0.0018	0.4884±0.0015	0.5119±0.0013	0.8072±0.0022	1.0717±0.0017
SR_2	0.2549±0.0012	0.3268±0.0011	0.4216±0.0015	0.4862±0.0014	0.5684±0.0013
SR_3	0.4657±0.0011	0.4484±0.0013	0.4727±0.0012	0.5515±0.0013	0.8191±0.0017
SR_4	0.3946±0.0017	0.4833±0.0021	0.7082±0.0014	0.8955±0.0012	0.9819±0.0014
SR_5	0.5811±0.0019	0.625±0.0018	0.7937±0.0016	1.0346±0.0017	1.5552±0.0015
BHA	0.8796±0.0017	0.9357±0.0011	1.1631±0.0029	1.2848±0.0018	1.3741±0.0031

The values are expressed as mean ± standard deviation (n=3).

Table 4: Average absorbance at 700 nm of different solvent extracts (hot) of *Stevia rebaudiana* and ascorbic acid for determination of reducing power

Extracts /Standard	Average absorbance at 700 nm at different concentration (µg/mL)				
	1	5	10	50	100
SR_6	0.2658±0.0019	0.3259±0.0025	0.4598±0.0014	0.5214±0.0012	0.5894±0.0014
SR_7	0.4299±0.0021	0.4951±0.0022	0.5455±0.0012	0.6783±0.0023	0.9498±0.0015
SR_8	0.4383±0.0015	0.4598±0.0013	0.5186±0.0017	0.7031±0.0027	0.9086±0.0018
SR_9	0.3513±0.0012	0.4043±0.0011	0.4664±0.0023	0.7025±0.0028	0.9972±0.0029
BHA	0.8796±0.0017	0.9357±0.0011	1.1631±0.0029	1.2848±0.0018	1.3741±0.0031

The values are expressed as mean ± Standard deviation (n=3).

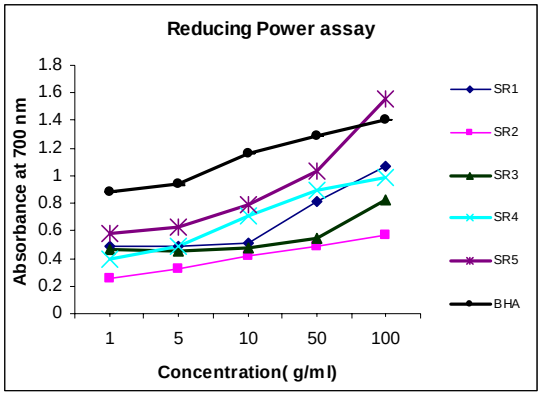

Figure 3: Reducing Power assay of 80% ethanol extract (room temp) and its different solvent fractions of *Stevia rebaudiana* and BHA (Butylated hydroxyanisole). SR_1: 80% ethanol extract; SR_2: n-Hexane soluble fraction of SR_1; SR_3: Dichloromethane soluble fraction of SR_1; SR_4: 1-Butanol soluble fraction of SR_1; SR_5: Water soluble fraction of SR_1.

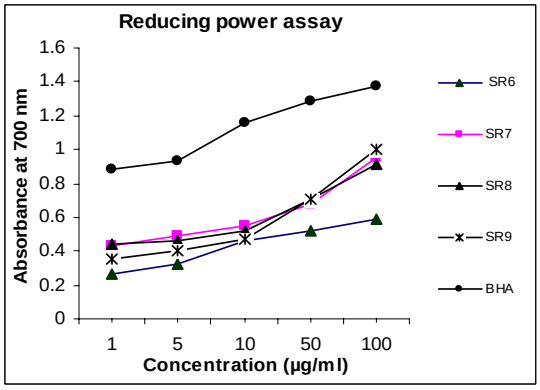

Figure 4: Reducing power assay of different solvent extracts (hot) of *Stevia rebaudiana* leaves and BHA (Butylated hydroxyanisole). SR_6: n-hexane extract; SR_7: dichloromethane extract; SR_8: methanol extract; SR_9: water extract.

Table 5: Total phenolic and total flavonoid concentrations of different extractives of *Stevia rebaudiana*

Extracts of *Stevia rebaudiana*	Total phenol	Total flavonoid
	mg of gallic acid equivalent per gm of dry extract	mg of quercetin equivalent per gm of dry extract
SR_1	65.21±0.97	125.64 ±1.07
SR_3	25.36±0.34	34.26±0.79
SR_4	51.26 ±0.48	82.23±0.64
SR_5	41.49 ±0.86	101.45±0.43
SR_7	15.33 ±0.78	23.56±0.89
SR_8	36.95±0.09	65.48±1.01
SR_9	31.25 ±0.25	76.94±0.35

The average values of three calculations are presented as mean ± SD (n=3)

DPPH free radical scavenging activity: A method based on the scavenging of the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively to predict the antioxidant activities of extracts of plants (Yen & Duh, 1994; Brand Williams *et al.*, 1995; Kulisic *et al.*, 2004). Free radical scavenging capacities of the different extracts and fractions of *S. rebaudiana* leaves measured by DPPH assay are shown in Table 1 and 2 and Figures 1 and 2. Inhibition values of 80% ethanol extract (SR_1) and its different solvent fractions, n-hexane (SR_2), dichloromethane (SR3), 1-butanol and water (SR_4) soluble fractions were found to be 95.59±0.714, 7.95±0.897, 41.67±0.486, 95.38±0.675 and 62.81±0.721 respectively at a concentration of 100µg/ml respectively compared to the inhibition value for ascorbic acid 96.32± 0.379 (Table 1, Figure 1). The inhibition values of hot extracts were found as 21.89±0.911, 26.47±,0.784 88.69±841 and 88.43±0.549, respectively when the leaf was successively extracted in soxhlet with n-hexane, dichloro methane, methanol and water compared to the inhibition value of standard (Ascorbic acid)

96.32± 0.743 at the concentration 100µg/ml. (Table 2, Figure 2). To scavenge DPPH free radicals IC_{50} values of 80% ethanol extract (SR1) and its 1-butanol soluble (SR_4) and water soluble (SR5), were found to be 8.02±0.874, 23.60± 0.760, 43.81±0.459 and Ascorbic acid .21± 0.92 4respectively (Table 1) and that for hot methanol and water extracts were observed as 44.61±0.821 and 23.70±0.711, respectively compared to the IC_{50} value 4.21± 0.924 of ascorbic acid, the standard compound (Table 2). Inhibition values for n-hexane and dichloromethane soluble fractions of 80% ethanol extract and hot n-hexane and dichloromethane extracts at 100µg/ml were less than 50%. According to the IC_{50} values in inhibition of DPPH radical, the activities of different extracts and fractions of *S. rebaudiana* leaves were observed in the as following order: SR_1>SR_4> SR_9> SR_8> SR_5.

Reducing power: A direct correlation between antioxidant capacity and reducing power of certain plant extracts has been reported (Tanaka *et al*, 1988). The reducing properties are generally associated with the presence of reductanes which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). The reducing power ability of all the extracts and fractions of *S. rebaudiana* was determined using BHA as positive control. In case of reducing power the higher the concentration of the test samples the higher the absorbance, the higher the absorbance the higher the inhibition. The maximum absorbance for 80% ethanol extract (SR_1) and its n-hexane (SR_2), dichloromethane (SR_3), 1-butanol (SR_4) and water soluble fractions (SR_5) were up to 1.0717±0.0017, 0.5684±0.0013, 0.8191±0.0017, 0.9818±0.0014 and 1.5552± 0.0015 respectively compared to ascorbic acid (1.374±0.0031) (Figure 3, Table 3). The maximum absorbance of the hot extracts, n-hexane (SR_6), dichloro methane (SR_7), methanol (SR_8) and water extracts (SR_9) were up to 0.5894±0.0014, 0.9498±0.0015, 0.9086±0.0018, 0.9972±0.0029, respectively compared to ascorbic acid (1.374±0.0031) (Figure 4, Table 4). The order of reducing power of different samples of *S. rebaudiana* was SR_5>SR_1>SR_9> SR_7> SR_4>SR_8>SR_3>SR_6>SR_2.

Total phenolicall concentrations: Generally, polyphenols all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Heinonen *et al.*, 1998; Parejo *et al.*, 2002; Lee *et al.*, 2003; Miliauskas *et al.*, 2004; Atoui *et al.*, 2005; Capecka *et al.*, 2005; Galvez *et al.*, 2005; Melo *et al.*, 2005). Total phenolic compounds amount in 80% ethanol extract (SR_1) and its n-hexane (SR_2), dichloromethane (SR_3), 1-butanol (SR_4) and water soluble fractions (SR_5) of *S rebaudiana* leaves were found to be 65.21±0.97, 25.36±0.34, 51.26±0.48 and 41.49±0.86 respectively (Table 5), whereas, the total amount of these compounds in hot extracts, dichloro methane (SR_7), methanol (SR_8) and water extracts (SR_9) were observed as 15.33±0.78, 36.95±0.09, 31.25±0.25 mg gm^{-1} gallic acid equivalent respectively (Table 5). The order of phenolic concentrations in different samples of *S. rebaudiana* leaves were SR_1> SR_4>SR_5>SR_8>SR_9>SR_3>SR_7.

The total flavonoids: Flavonoids, commonly found in plants have been reported to have significant antioxidant activity (Vinson *et al.*, 1995). The total flavonoids concentration of 80% ethanol extract (SR_1) and its n-hexane (SR_2), dichloromethane (SR_3), 1-butanol (SR_4) and water soluble fractions (SR5) fractions were found to be 125.64±1.07, 34.26±0.79, 82.23±0.64 and 101.45±0.43, respectively (Table 5) and that for hot extracts, dichloro methane (SR_7), methanol (SR_8) and water extracts (SR_9) were observed as 23.56±0.89, 65.48±1.01, and 76.94±0.35 mg/gm quercetin equivalent per gm of dry extract, respectively (Table 5). The amount of total flavoinds in different samples of *S. rebaudiana* leaves were in the order of SR_1>SR_5> SR_4>SR_9>SR_8>SR_3>SR_7.

The significant inhibition value of 80% ethanol extract, its 1-butanol & water soluble fractions and hot methanol and water extracts of *S rebaudiana* leaves might be due to the presence of significant amount of different types of polyphenolic compounds including flavonoids in these samples.

Conclusion

From the observed results of antioxidant study following four different methods it can be concluded that 80% ethanol extract and its 1-butanol and water soluble fractions, and hot methanol and water extract of *S. rebaudiana leaves* from Bangladesh possess significant antioxidant acitvity. The results obtained in this study clearly indicate that the leaves of *Stevia rebaudiana* from Bangladesh has a significant potential to be used as a natural antioxidant.

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