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COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY OF METHANOLIC AND ETHANOLIC EXTRACTS OF *STEVIA REBAUDIANA* BERTONI

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ABSTRACT

In the modern word it has been realized the herbal drugs strengthens the body system specifically and selectively without side effects. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). *Stevia rebaudiana* Bertoni, belonging to the family Asteraceae, is a perennial sweet herb. It is a native medicinal plant of Paraguay and is a new alternative source of calorie-free sweetener having no carbohydrates. From the observed results of antioxidant study following 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 *Stevia rebaudiana* leaves possess significant antioxidant activity.

Keywords: *Stevia rebaudiana*, Antioxidant activity, Methanolic and Ethanolic extracts etc.

INTRODUCTION

Antioxidants work by bringing under control the rogue and unstable oxygen molecules that have an odd number of electrons. These oxygen molecules known as free radicals are highly reactive; they attack cells, DNA, and protein thereby accelerating the aging process. The antioxidants work in harmony and the efficacy of one antioxidant depends upon the availability and concentration of another. Essentially, antioxidants work by donating an electron to the unstable free radical. This stabilizes the free radical and converts it into a harmless compound that may safely be removed from the body.[1] Oxidation reactions occur when life essential oxygen combusts within the human body and produces by-products referred to as oxygen free radicals which steal electrons from other molecules, like

proteins, lipids and DNA, causing damage. In case of DNA, the problem intensifies and genetic cell mutations may occur which may become a common cause of cancer. Uninhibited over time, free radical damage. Which are formed in the body, thus causing aging. An overload of free radicals has been linked to certain diseases, including heart disease, liver disease and some cancers.[2,3]

Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals $O_2^{\cdot}$ and hydroxyl radicals (OH·), as well as non-free radicals ($H_2O_2^{\cdot}$) and singlet oxygen. In the body, free radicals are derived from two sources: endogenous sources e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc.[4,5,6]

In search of novel sources of antioxidants in last years, medicinal plants have been extensively studied for their antioxidant activity. From ancient times, herbs have been used in many areas, including nutrition, medicine, flavoring, beverages, cosmetics etc. The ingestion of fresh fruit, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases. The higher intake of plant foods correlates with lower risk of mortality from these diseases. Approximately 60% of the commercially available anti-tumoral and anti-infective agents are of natural origin. Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, met-al chelators and reductants of ferryl hemoglobin.[7, 8, 9, 10]

Stevia rebaudiana a non-caloric substitute to conventional sucrose, distinctly possesses good antioxidant activities and thus has the ability to boost the immune system and prevent free radical mediated diseases. It contains micronutrients like selenium, zinc, manganese which play an important role as antioxidants. It is a small perennial shrub growing up to 1m tall and with leaves 2-3cm long and native to regions of Paraguay and Brazil. It is popular as the "sweet herb of Paraguay".[11, 12, 13]

MATERIALS AND METHODS

Plant Collection and Extract Preparation

The selected medicinal plant was *Stevia rebaudiana* was taxonomically identified and were collected from Department of Aromatic and Medicinal plant, Agriculture College, Rewa, M. P. Plant parts viz., stems, roots, leaves and flowers were shade dried, grounded and pass through sieve no. 80. Leaves dried at room temperature and coarsely ground before extraction. Each part was extracted by maceration method and ethanol as solvent. The uniform particle sized powder was packed in thimble and extracted in soxlate apparatus with methanol till the solution in the thimble becomes clear. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal. It was then stored in refrigerator below 10^0 C for further use.

Preliminary Phytochemical Screening and Determination of Phytoconstituents

Phytoconstituents in herbal medicine are important to oxidative stress resulted due to certain pathological conditions. Therefore, it is necessary to estimate amount of the major class of the constituents present in the crude extract. The methanolic extracts were subjected to preliminary phytochemical screening to judge the presence of phytoconstituents as per the method.

Chemicals, Regents and Buffer

Disphenyl-2-2 picryl hydrazyl (DPPH), FolinCiocolteu's reagents, (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 were purchased from SD Fine chemicals, India. Naphthyl ethylene diamine dihydrochloride and all chemicals used were of analytical grade.

Equipment

UV/VIS spectrophotometer (Sistronic 135, Mumbai) India.

Preparation of Test and Standard Solutions

The extracts and the standard antioxidants, ascrobic acid and rutin, were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for *in-vitro* antioxidant study. The stock solutions were serially diluted with DMSO to get required dilutions.

Determination of Total Phenolic Content by Folin-Ciocalteu Reagent

Total phenol estimation was carried out with folin-ciocalteu reagent (FCR) method. 5 mg of the sample was weighed and dissolved in 1ml of 50% methanol using a vortex mixer (touch type) followed by adding to it 4 ml of 50% methanol and finally mixing through sonication to prepare a sample of concentration 1mg/ml. 0.5 ml of this solution was pipetted out in a test tube to which was added 3.5 ml of distilled water followed by addition of 0.25 ml of Folin-ciocalteu reagent (FCR).

This was left for incubation for 1-8 minutes at room temperature. Lastly, to this was added 0.75 ml of 20% sodium carbonate solution and the final sample solution in the test tube was left to incubate for 2 hours. The sample was prepared in duplicate. Finally the absorbance was measured at 765 nm against a reagent blank. The procedure was repeated for all the five varieties of *Stevia rebaudiana*. The standard curve was obtained using gallic acid monohydrate. The total phenol content was expressed as gallic acid equivalent to % w/w of the extracts.[14]

Determination of Total Flavonoid Content by Aluminium Chloride Colorimetric Method

The total Flavonoids content of the extracts was determined by aluminium chloride method. 10 mg of the sample was weighed and dissolved in 1ml of 80% ethanol using a vortex minex (Touch Type) followed by adding it to 80% ethanol and finally mixing through sonication to prepare a sample of concentration 1mg/ml. 0.5 ml of this solution was pipetted out in a test tube to which was added 1.5 ml of methanol, 0.1 ml and 2.8ml of distilled water. A yellow color indicated the presence of flavonoids. The final sample solution in the test tube was left to incubate for 30 minutes at room temperature.[15]

The sample was prepared in duplicate. Finally, the absorbance was measured at 415nm against a reagent blank. The procedure was repeated for all the five varieties of S. rebaudiana. The standard curve was obtained using quercetin using solution in the range of 1-10 ug/ml as shown in fig. 2. The results were expressed as mg quercetin/g dry weight by comparison with quercetin standard curve, which was made under the same conditions.

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of the different methanolic extracts was evaluated using the method of Miliausk et al., 2004.[16] 10 mg of the sample was weighed and dissolved in 1ml of

methanol using a vortex mixer (Touch Type) followed by adding to it 19 ml of methanol and finally mixing through sonication to prepare a sample of concentration 0.5 mg/ml. DPPH solution was prepared by dissolving 4mg of DPPH in 100 ml of methanol. Various dilutions of the sample were prepared with methanol resulting in concentrations 5µg/ml, 10µg/ml., 20µg/ml, 30µg/ml. 40µg/ml. 50 µg/ml, 100µg/ml, and 150 µg/ml. To each dilution was added 2 ml of the prepared DPPH solution in methanol. A control was also prepared simultaneously consisting of 2 ml methanol and 2ml DPPH solution. The prepared dilutions were then left for color development in the dark for 20 minutes. Finally, the absorbance was measured at 517 nm against a reagent blank. The procedure was repeated for all the five varieties of *Stevia rebaudiana*. The standard curve was obtained using ascorbic acid. A plot of concentration vs/ the percentage inhibition of DPPH radical gave the IC50 value which is the concentration of sample required to inhibit 50% of DPPH radical.

Tannins

7.5 ml of distilled water was added to 100µl of sample. After that 0.5 ml of Folin denis Reagent (FDR), followed by 1ml of 35% Na2CO3 were added. The final volume was made up to 10ml with distilled water. The blue color produced was measured spectrophotometrically at 700 nm. The calibration curve was prepared using Tannic acid, and expressed in mg/g dry weight.

Enzymatic Assay

Superoxide dismutase Activity

This method relies on the reduction of Nitroblue tetrazolium (NBT). 1 ml of sodium carbonate, 0.4 ml NBT, 0.2 ml EDTA was added to 0.5 ml plant extract. The reaction was initiated with the addition of 0.4 ml of Hydroxylamine hydrochloride. Zero absorbance was taken at 560 nm using spectrophotometer followed by recording the absorbance after 5 minutes at 25^0C. Units of SOD were expressed a amount of enzyme required for inhibiting the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of protein.[17]

Catalase

To 1ml of plant extract, 5ml of 300 µm phosphate buffer (pH 6.8) containing 100µM Hydrogen peroxide was added and left at 25^0 C for a minute. 10ml of 2% sulphuric acid was added to arrest the reaction, and the residual H_20_2 was tirated with potassium permanganate

(0.01N) till pink color was obtained. Units of enzyme activity were expressed as ml of 0.01 N potassium permanganate equivalent of hydrogen peroxide decomposition per min per mg of protein.[18]

Peroxidase Activity

3.5 ml of phosphate buffer, pH 6.5, was taken in a clean dry curvette, 0.2 ml of plant extract and 0.1 ml of freshly prepared O-dianisidine solution was then added to it. The temperature of assay was brought to 28-30^{o} C, and absorbance was recorded at 430 nm. Then 0.2 ml of 0.2M hydrogen peroxide sec interval up to 3min. A graph was plotted with increase in absorbance against time. The enzyme activity was expressed per unit time per mg of protein.[19]

Antioxidant Ability Assay

Determination of Total Antioxident capacity 33

This assay is based on the reduction of Mo (VI) to Mo (V) by the extracts with the subsequent formation of a green phosphate complex/Mo (V) at acidic pH. An aliquot of 0.3ml of each extract was combined with 3ml of reagent mixture (0.6 M sulphuric acid, 28mM sodium ph0osphate and 4mM ammonium molybdate) in appendroff tube, and incubated at 95^{0}C for 90 minutes. The absorbance of the extracts was measured at 695nm after cooling them to room temperature. The antioxidant activity is expressed at the number of gram equivalent of ascorbic acid.[20]

ABTS Free Radical Scavenging Assay

Total antioxidant status of the extracts was measured using 2, 2'azinobis [3ethylbenzthiazoline]-6suffonic acid (ABTS) assay. The assay relies on the antioxidant ability of the samples to inhibit the oxidation of ABTS to ABTS radical cation. ABTS radical cations were produced by the reaction of aqueous ABTS (7mM) with 2.45 mM potassium persulfate. The reaction mixture was left to stand at room temperature overnight (12-16 hrs) in dark before use. The resultant intensely coloured ABTS radical cation was diluted with 0.01 M, pH .4 PBS (phosphate buffered saline) to give absorbance of -0.70 at 734nm. The plant extracts were diluted 100 times with the ABTS solution to a total volume of 1ml. The absorbance was measured at time interval of 1 min after addition of each extract. The assay was performed in triplicate. The total antioxidant activites were expressed as mM trolox equivalent antioxidant capacity (TEAC).[21]

Determination of Reducing Power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (11). Reducing power of *Stevia rebaudiana* methanolic and ethanolic extracts was and standard solutions of butyrate hydroxyl toluene (BHT, 100-1000µg/ml) were prepared in 1ml of the prepared concentrations. 2.5ml of potassium ferricyanide, 2.5 ml of phosphate buffer (pH6.6, 0.2m) were added and this mixture incubated at 50^0C for 20 minutes. A portion (2.5ml) of tichloroacetic acid (10%) was added to the mixture. Then, it was centrifuges for 10 min at 300rpm. The upper layer of the solution (2.5ml) was with 2.5ml of distilled water and 0.5 ml of ferric chloride (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butyrate hydroxyl toluene was used as standard.

Scavenging of Hydrogen Peroxide

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et.al. Hydrogen peroxide (40mM) was prepared in phosphate buffered (pH 7.4). Standard (ascorbic acid) and extract solutions were prepared at concentrations of 20, 40, 60, 80, 100µg/ml in distilled water. To 3.4 ml of standard or extract solutions 0.6 ml of hydrogen peroxide solution was added. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm. The percentage of where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extracts and standard.[22]

Statistical Analysis

The data are expressed as Mean ± Standard Deviation (S.D.) from triplicate determination. Linear regression analysis was used to calculate IC50 values whenever needed. Data were analyzed by one way analysis of variance (ANOVA) comparison test with equal sample size. The difference was considered significant when p value < 0.05.

RESULTS AND DISCUSSIONS

The result of the preliminary phytochemical screening was carried out on the metabolic extracts of and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides (anthraquinone, cardiac and coumarin), saponins, carbohydrates, flavonoids, tannins, amino acids, steroids, waxes supporting the reason for its wide range of

biological activities as showed in Table 1. The finding suggested that most of the constituents are present.

The current study also aimed at finding out the variety of *Stevia rebaudiana* with the highest content of rebaudioside Among the five varieties procured from different locations, but except a single location on any other sites and varieties of experimental plants were available in the Rewa city. The work was targeted with a single species. It also focused on finding out the present variety with highest antioxidant potential which can fight against various oxidative stresses in the human body and hence included a comparative evaluation of antioxidant activity. The climatic conditions like temperature, rainfall, humidity as well as solid conditions, altitude etc that vary from region to region as well as the time of collection may be responsible for such a variation in the glycosidal content of rebaudioside. But in the Rewa city the whole plant was cultivated temperature and other climatic parameters were associated with plants. Folin flavonoid content was also determined through aluminium chloride colorimetric assay which justify the presence of aromatic nature of the plants and shown oil% including shown coloring behavior along with used organic solvent.

Free radicals and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to various diseases like diabetes, cancer, etc. Many constituents of plants have antioxidant activity and serve as a protective agent against the damage caused by such diseases.

In the present study, Phenolic content in the methanolic root extract of *Stevia rebaudiana* was found to be higher than that of other parts. The order of phenolic concentration in different extracts of S. rebaudiana was root>leaves> stem>flower. Phenolic compounds have been reported with multiple biological effects including antioxidant activity. Anti-oxidative property of these polyphenols is due to their high reactivity as hydrogen or electron donor. Polyphenols have the ability to stabilize and delocalize the unpaired electron, and have the potential to chelat metal ions. Flavonoids and Tannins are also known to possess antioxidant activity. In Flavonoids, this is due to certain mechanisms such as free radical 470 scavenging, chelation of metal ions viz., iron and copper, and inhibition of enzymes responsible for free radical generation. Depending upon the structure, flavonoids are able to scavenge practically all known reactive oxygen species. The pattern of amount of tannins and flavonoids observed in plant extracts was Leaf>root>stem<flower and leaf<stem<root<flower respectively.

Biological system counterpoised the inevitable generation of ROS by selection of enzymatic defense system. These enzymatic antioxidants SOD, catalases, peroxidase, plays an important role in protecting cell wall against the lipid per oxidation and other cellular damage. SOD protects cell wall by catalyzing superoxide ions into H_2O and H_2O_2, Catatlase works at high concentration of H_2O_2 and it readily detoxify it into H_2O and O_2.

Peroxides catalyze the degradation by oxidizing glutahions with the formation of its conjugates 44. The levels of enzymatic antioxidants are collectively compiled in Table 2. The highest activity of SOD, catalase and peroxidise was observed in root, depicting its higher antioxidative potential.

A DPPH: free radical scavenging assay was used to evaluate the antioxidant activity showed the maximum potency of activity with an IC50 of 54μg/ml (Table 4) and hence, this can serve as a source for curbing various health problems related to oxidative damage. As these compounds contribute to the antioxidant efficacy of natural products hence this observations can be directly correlated to the leaves showing the most potent antioxidant activity.

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radicals, the delocalization of the gained electron over the phenolic antioxidant occurs, and the stabilization by the resonance effect of the aromatic nucleus, which prevents the continuation of the free radical chain reaction (table-2and 3).

In Flavonoids, this is due to certain mechanisms such as free radical scavenging, chelation of metal ions viz., iron and copper, and inhibition of enzymes responsible for free radical generation. Depending upon the structure, flavonoids are able to scavenge practically all known reactive oxygen species. The pattern of amount of tannins and flavonoids observed in plant extracts was leaf>root>stem>flower and leaf>stem<root<flower respectively. Plant contains different antioxidants, and it is difficult to measure each antioxidant separately.
Therefore several methods have been developed to measure the antioxidant activity. ABTS, DPPH and total scavenging ability assays were used to measure scavenging activity of different extracts of plant parts. It was observed that there was a difference in antioxidant activity of different extracts of plant *Stevia rebaudiana*. Root and leaf extracts of *Stevia rebaudiana* at a concentration of 800µg/ml DPPH, exhibited equally high radical scavenging activity of 82.36% and the least of 47.05% by the flower extract. Steady state absorbance value for pure ascorbic acid and BHA (reference sample) was 0.19 and 0.25, exhibiting the highest antioxidant Plant contains different antioxidants and it is difficult to measure each antioxidant separately. Therefore several methods have been developed to measure the antioxidant activity. ABTS, DPPH and Total scavenging ability assays were used to measure scavenging activity of different extracts of plant parts. It was observed that there was a difference in antioxidant activity of different extracts of plant *Stevia rebaudiana*.

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Free radical Disphenylpicrylhydrazyl (DPPH) Antioxidant Activity

DPPH is a stable introgen centred freeradical, upon reduction either by hydrogen or electron donation, its colour changes from violet to yellow. The anti-oxidant potential of the extract is based on the determination of the concentration of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) at steady state in a methanol solution after adding the plant extracts. DPPH absorbs at 51nm,

and its concentration is reduced the initial DPPH concentration was evaluated. The DPPH radical-scavenging activity in terms of percentage was calculated [35,36] according to the following equation: DPPH scavenging activity (%) = {1-(Abs 515 sample/Abs515 DPPH solution)} x 100% (table 4 and 5).

Total Phenolic Concentrations

Generally, polyphenols all share the same chemical patterns, one or more phenolic groups. Total phenolic compounds amount in 80% ethanol extract (SR_1) and its n-hexane (SR_2), dichloromethane (SR3), 1-butanal (SR4) and water soluble fractions (SR5) of *Stevia rebaudiana* leaves were found to be 63.21±0.97, 27.36±0.34, 50.26±0.48 and 45.49±0.86 respectively, whereas the total amount of these compounds in hot extracts, dichlore methane (SR7), methanol (SR8) and water extracts (SR9) were observed as 17.33 ±0.78, 38.95±0.09, 30.25±0.25 mg gm-1 gallic acid equivalent respectively (table 6). The order of phenolic concentrations in different samples of S. rebaudiana leaver were SR1>SR4>SR5>SR8>SR9>SR3>SR7.

The Total Flavonoids

Flavonoids, commonly found in plats have been reported to have significant antioxidant activity. The total flavonoids concentration of 80% ethanol extract (SR1) and its n-hezane (SR2), dichloromethane (SR3), 1-butanol (SR4) and water soluble fractions (SR5) fractions were found to be 122.34±1.07, 30.26±0.79, 80.23±0.64 and 100.45±0.43, respectively and that for hot extracts, dischloro methane (SR7), methanol (SR8) and water extracts (SR9) were observed as 20.56±0.89, 63.48±1.01, and 75.94±0.35 mg/gm quercetin equivalent per gm of dry extract, respectively. The amount of total flavoinds in different samples of *Stevia rebaudiana* leavers were in the order of SR1> SR5>SR4>SR9>SR8>SR3>SR7. 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 (table-6).

ABTS radical scavenging and Total antioxidant ability assay showed the similar trend of antioxidative potential in methanolic extracts of *Stevia rebaudiana* i.e., Root>leaf>stem>flower. Higher phenol content has direct correlation with high radical

scavenging activity. Thus, it can be concluded that root extract having greater phenolic content, have higher enzymatic antioxidants and thus highest antioxidant activity.

Table: 1 Preliminary phytochemical screening of the methanolic extracts of *Stevia rebaudiana.*

S. NO.	Phytochemicals	Presence/Absence
1	Alkaloids	+ve
2	Saponins	+ve
3	Carbohydrates	+ve
4	Glycosides (general)	+ve
5	Anthraquinone glycosides	+ve
6	Cardiac glycosides	+ve
7	Coumarin glycosides	+ve
8	Cyanogenetic glycosides	-ve
9	Tannins	+ve
10	Proteins	-ve
11	Waxes	+ve
12	Flavonoids	+ve
13	Amino acids	+ve
14	Acidic compounds	-ve

Table: 2 Total phenols, flavonoids and tannins of methanolic extracts of various parts of *Stevia rebaudiana.*

S. NO.	Plant parts	PHENOLS (mg GAE)	TANNINS (mg TAE)	FLAVONOIDS (% Quercetin)
1	Root	15.75±0.45	13.56±6.78	16±1.86
2	Stem	5.5±2.37	8.21±3.95	3.53±2.44
3	Leaf	12.40±5.52	3.43±2.45	4.73±0.38
4	Flower	6.93±3.16	11.98±1.37	1.73±0.83

Values are mean ± SD, values are significantly different at $p<0.05$.

Table: 3 Enzymatic antioxidant levels of different parts of *Stevia rebaudiana* Bertoni.

S. NO.	Plant Parts	SOD (µ/mg)	Catalase (µ/mg)	Peroxidases (µ/mg)
1	Root	3.44±0.12	6.68±0.35	3.42±1.05
2	Stem	4.45±0.26	4.33±0.25	2.22±0.45
3	Leaf	2.87±0.22	5.3±0.36	1.56±0.23
4	Flower	2.32±0.25	2.06±0.37	1.46±1.24

Table: 4DPPH free radical scavenging activity of 80% ethanol extract (at room temp.) of *Stevia rebaudiana.*

Extracts/fractions/ Standard	% Inhibition at different conc. (µg/ml)					IC_{50} (µg/ml)
	1	5	10	50	100	
SR_1	25.70±0.25	32.05±0.42	62.86±0.21	92.38±0.55	93.534±0.56	7.02±0.77
SR_2	1.50±0.85	1.01±1.45	4.52±0.25	3.41±0.52	5.35±0.68	---
SR_3	1.88±0.92	4.08±0.42	15.09±0.426	22.46±0.23	40.67±0.48	----
SR_4	1.22±0.78	5.82±0.85	35.82±0.75	84.66±0.41	97.38±0.67	25.06±0.21
SR_5	2.33±0.45	6.67±0.68	23.06±0.17	57.25±0.53	65.81±0.72	45.81±21
AsA	10.88±0.53	55.97±0.35	95.23±0.56	97.25±0.24	95.32±0.37	4.23±0.81

Table: 5 DPPH free radical scavenging activity of different solvent extract (hot) of *Stevia rebaudiana,*

Extracts/fractions/ Standard	% Inhibition at different conc. (µg/ml)					IC_{50} (µg/ml)
	1	**5**	**10**	**50**	**100**	
SR_6	1.22±0.35	12.23±0.65	15.53±0.42	22.22.±0.69	24.69±0.55	---
SR_7	4.14±0.38	15.5±0.68	19.27±0.67	25.56±0.54	25.43±0.32	---
SR_8	6.32±0.54	13.27±0.54	37.34±0.16	55.20±0.29	90.16±0.54	45.32±0.54
SR_9	6.10±0.15	13.65±0.45	33.12±0.45	84.90±0.34	90.67±0.15	25.17±0.72
AsA	11.08±0.45	55.18±0.54	97.56±0.25	96.34±0.43	95.32±0.56	5.56±0.67

Table: 6 total phenolic and total flavonoids concentrations of different extract of *Stevia rebaudiana.*

S. No.	Extract of *Stevia rebaudiana*	Total phenol	Total flavonoids
		mg of gallic acid equivalent per gm of dry extract	mg of quercetin equivalent per gm of dry extract
1	SR_1	63.21±0.97	122.64±1.07
2	SR_3	27.36±0.34	32.26±0.79
3	SR_4	50.26±0.48	80.23±0.64
4	SR_5	45.49±0.86	100.45±0.43
5	SR_7	17.33±0.78	20.56±0.89
6	SR_8	38.98±0.09	63.48±0.10
7	SR_9	30.25±0.25	75.94±0.35

CONCLUSION

Stevia sweetener extractives have been suggested to exert beneficial effects on human health, including antihypertensive, antihyperglycemic and anti-human rotavirus activities. From above study we can conclude that Stevia also show higher free radical scavenging activity. Therefore, micropropagated and field-adapted plants of *Stevia rebaudiana* can be used as a rich source of natural antioxidants also.

REFERENCES

1. Shahidi, F. and P.D. Wanasundara,. Phenolic antioxidants, Cri. Rev. Food Sci. Nutr., 1992; 32: 67-103.
2. Buyukokuroglu, M. E., Gulcin, I. Oktay M. and Kufrevioglu, O. I. In-vitro antioxidant properties of dantrolene sodium. Pharmacol, Res., 2001; 44: 491-494.
3. Melis, M. S. Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: Renal effects. Journal of Ethnopharmacology, 1995; 47: 129 –134.
4. Dao, K. N., & Le, V. H. Biological properties of flavonoids from *Stevia rebaudiana* Bert.Tap Chi Duoc Hoc, 1995; 2: 17 –21.
5. Mizutani, K., & Tanaka, O. Stevia, the genus Stevia. Medicinal and aromatic plants — industrial pro files. In A. D. Kinghorn (Ed.), Use of *Stevia rebaudiana* sweeteners in Japan, 2002; 19: 178 – 195. London: Taylor and Franc is.
6. Mohamed, A. A. F., & Chang, Y. L. Optimization of phenolic and dietary fibre extraction from date seeds. Food Chemistry, 2008; 108: 977 – 985.
7. Curi, R., Alvares, M., Bazotte, R. B. L. M., Botion, J., Godoy, L., & Bracht, A. Effect of *Stevia rebaudiana* on glucose tolerance in normal adult humans. Brazilian Journal of Medical and Biological Research, 1986; 19: 771 – 774.
8. Wu, S., & Ng, L. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. abbreviata Ser.) in Taiwan. LWT- Food Science and technology, 2008; 41: 323 –330.
9. Alvares, M., Bazzone, R. B., Godoy, G. L., Cury, R., & Botion, L. M. Hypoglycemic effect of *Stevia rebaudiana* Bertoni. First Brazilian Seminar on Stevia rebaudia na, 1981; 13: 1. Campinas, Brazil: Instituto Tecnologico Alimet.
10. Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M., & Morelli, I. Antioxidant principles from Bauhinia terapotensis. Journal of Natural Products, 2001; 64: 892 –895.
11. Chang, C., Yang, M., Wen, H. and Chern, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Analaysis, 2002; 10: 178-182.
12. Kinghorn, A. D. Food ingradient safety review. *Stevia rebaudiana* leaves, 1992; 16: 3. (unpublished report submitted to Uropean commission)
13. Kulisic, T., Radonic, A., Katalinic, V. M. Use of different methods for test in gantioxidative activity of oreganoessential oil, Food Chem, 2004; 85: 63 3– 640.
14. Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P. and Glower W. Phenolic

compounds and their role in oxidative processes in fruits. *Food Chem*., 1999; 66: 401–436.

15. Yen, G. C. and Duh, P. D. Scavenging effect of methanolic extracts of peanut hulls on free -radical and active oxygen species, J. Agric. and Food Chem., 1994; 42 : 6 29– 632.
16. Miliauskas, G., Venskutonis, P. R. and Van Beek, T. A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chem., 2004; 85: 231- 237.
17. Melo, E. A., Filho, J. M. and Guerra, N. B. Characterization of antioxidant compounds in aqueous coriander extract (*Coriander sativum* L.), Lebensm-Wiss. Technol., 2005; 38: 15-19.
18. Kalpana M., Anbazhagan, M., Natarajan V. and Dhanavel D. Improved micropropagation method for the enhancement of biomass in *Stevia rebaudiana* Bertoni, *Recent Research in Science and Technology,* 2010; 2(1): 008–013.
19. Kim. I. S., Yang, M., Lee, O. H. 2011.The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT Food Sci Tech 44: 1328–1332.
20. Siddhuraju, P., Becker, K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) Seed extracts. Food Chem, 2007; 101: 10–19.
21. Tadhani, M. B., Patel, V. H., Subhash, R. In vitro antioxidant activity of *Stevia rebaudiana* leaves and callus. J Food Comp Anal, 2007; 20: 323–329.
22. Megeji, N. W., Kumar, J. K., Singh, V. Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. Curr Cell Sci, 2005; 88: 801–80.