Experimental and Toxicologic Pathology 64 (2012) 807–811

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert

Shruti Shukla[a,*], Archana Mehta[b], Pradeep Mehta[b], Vivek K. Bajpai[c]

[a] Department of Food Science and Technology, Yeungnam University, 214-1 Dae-dong, Gyeongsan-si, Gyeongsangbuk-do 712-749, Republic of Korea
[b] Faculty of Life Sciences, Dr. H. S. Gour University, Sagar 470003, Madhya Pradesh, India
[c] Department of Biotechnology, Yeungnam University, 214-1 Dae-dong, Gyeongsan-si, Gyeongsangbuk-do 712-749, Republic of Korea

ARTICLE INFO

Article history:
Received 16 September 2010
Accepted 8 February 2011

Keywords:
Stevia rebaudiana
Antioxidant activity
Aqueous leaf extract
Total phenolic content

ABSTRACT

In the present study, we carried out a systematic research on relative antioxidant activity of aqueous leaf extract of *Stevia rebaudiana*. The DPPH activity of aqueous leaf extract (20, 40, 50, 100 and 200 μg/ml) was increased in a dose dependent manner, which was found in the range of 40.00–72.37% as compared to ascorbic acid 64.26–82.58%. The IC_{50} values of aqueous extract and ascorbic acid in DPPH radical scavenging assay were obtained to be 83.45 and 26.75 μg/ml, respectively. Measurement of total phenolic content of the aqueous leaf extract of *S. rebaudiana* was achieved using Folin-Ciocalteau reagent containing 56.73 mg/g of phenolic content, which was found significantly potent when compared to reference standard gallic acid. The aqueous extract also inhibited the hydroxyl radical, nitric oxide and superoxide anions with IC_{50} values of 100.86, 98.73 and 100.86 μg/ml, respectively. The greater amount of phenolic compounds leads to more potent radical scavenging effects as shown by the aqueous leaf extract of *S. rebaudiana*.

1. Introduction

Approximately 80% of the world populations depend exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual however in the recent years, more and more people are complementing their treatment with natural supplements (Dursum et al., 2004). Nowadays motivation of people towards herbs is increasing due to the concern about the side effects of synthetic chemical drugs. People want to concern their own health rather than submitting themselves to impersonal health care system. Many herbal and some common medicinal plants are good sources of antioxidant compounds. Many of the biologically active substances found in plants, including phenolic compounds (flavonoid, phenolics) are known to possess potential antioxidant properties. The antioxidant activity of medicinal plants depends on the concentration of individual antioxidant entering into the composition (Larson, 1988).

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals. Free radicals have been implicated in the etiology of several major human ailments including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis (Devasagayam et al., 2004). Antioxidants have been reported to prevent oxidative damage caused by free radical, they can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers (Buyukokuroglu et al., 2001). The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body which are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids lipoproteins and DNA (Farber, 1994). This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases as well as ageing process.

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Natural plant-based antioxidants especially phenolics and flavonoids have been exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu et al., 2000). However there is still a demand to find more information concern-

* Corresponding author. Tel.: +82 53 810 3590; fax: +82 53 810 4662.
E-mail address: shruti.shukla15@yahoo.com (S. Shukla).

0940-2993/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.etp.2011.02.002

ing the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Stevia rebuadiana (Bert.), Bertoni is an herbaceous perennial plant of the Asteraceae family. It is native of Paraguay, where it grows wild in sandy soil (Goenadi, 1983). The main sweet component in the leaves of *S. rebuadiana* is stevioside (Geuns, 2000). Stevia sweetener extractives have been suggested to exert beneficial effects on human health, including antihypertensive (Chan et al., 2000), antihyperglycemic noncariogenic, anti human rota virus activities, glucose metabolism (Suanarunsawat and Chaiyabutr, 1997) and renal function (Jutabha and Chatsudthipong, 2000). Aqueous extract of *S. rebuadiana* dried leaves induce systemic and renal vasodilation, causing hypotension, diuresis and natriuresis in rats (Melis, 1995).

To the best of our knowledge, no reports are available on the antioxidant potential of aqueous extract of *S. rebuadiana* leaves therefore, present investigation was undertaken to examine the total phenolic content and antioxidant potential of aqueous leaf extract of *S. rebuadiana* through various *in vitro* models.

2. Materials and methods

2.1. Chemicals

Chemical reagents nitroblue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Germany, gallic acid (standard solution) (Loba Chemie, Mumbai), sodium carbonate (S.D-Fine Chemicals, Mumbai) and sodium nitroprusside (10 mM) solution and trichloro acetic acid (TCA) (S.D-fine chemicals, Mumbai).

2.2. Plant material

The leaves of *Stevia rebaudiana* were collected in March 2007 from Sagar District, Madhya Pradesh, India. Further taxonomic identification was conducted by harberium incharge at the Department of Botany, Dr. H. S. Gour University, Sagar, MP, India. A voucher specimen (Bot/H/3352) was deposited in the herbarium of Laboratory of Microbiology, Department of Botany, Dr. H. S. Gour University, Sagar, MP, India.

2.3. Preparation of the extract

The air-dried leaves of *S. rebaudiana* (50 g) were powdered and then extracted with 500 ml of distilled water by merceration process. The crude extract was filtered and evaporated under reduced pressure to give a viscous mass with a percentage yield of 8.0% (w/w). The extract was stored at 4 °C for further use. This aqueous leaf extract of *S. rebaudiana* (ALES) was reconstituted in known amount of water and used for the assessment of antioxidant activity.

2.4. Determination of total phenolic content

Total soluble phenolics in the leaf aqueous extract of *S. rebaudiana* were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound (Slinkard and Singleton, 1977). 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later, 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was measured at 760 nm. The concentration of total phenolic content was expressed as mg/g of dry extracts. The concentration of total phenolic content in the extract was determined as μg of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

$$\text{absorbance} = 0.0008 \times \text{gallic acid } (\mu g).$$

2.5. Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity

The DPPH free radical scavenging activity of aqueous extract of *S. rebaudiana* leaf was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Blios, 1958). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (20–200 μg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference standard compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. DPPH radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\%\text{inhibition} = \frac{A_0 - A_t}{A_0} \times 100.$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

2.6. Determination of hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was measured using modified method as described previously (Halliwell et al., 1987). Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of extract (20–200 μg/ml) each dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in a sequence. The mixture was then incubated at 37 °C for 1 h. 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of (10%) TCA and 1.0 ml of (0.5%) TBA (in 0.025 M NaOH containing 0.025 M NaOH BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract was measured as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

$$\%\text{inhibition} = \frac{A_0 - A_t}{A_0} \times 100.$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the sample of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control.

2.7. Determination of nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Marcocci et al., 1994) which interacts with oxygen to produce nitric ions that can be estimated by using greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate buffer

saline (PBS) was mixed with 3.0 ml of different concentrations (20–200 μg/ml) of the ALES and incubated at 25 °C for 150 min. The samples were added to greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamme was measured at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Greiss reagent as a positive control. The percentage of inhibition was measured by the following formula:

$$\%\text{inhibition} = \frac{A_0 - A_t}{A_0} \times 100.$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

2.8. Determination of superoxide radical scavenging activity

This activity was measured using NBT (nitro blue tetrazolium reagent) method as described by Sabu and Ramadasan (2002). The method is based on generation of superoxide radical (O_2^-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, which gets reduced to nitrite. Nitrite in presence of EDTA gives a color that was measured at 560 nm. Test solutions of extract (20–200 μg/ml) were taken in a test tube. To this, reaction mixture consisting of 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube and immediate reading was taken at 560 nm. 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction then reaction mixture was incubated at 25 °C for 15 min and reduction of NBT was measured at 560 nm. Ascorbic acid was used as the reference compound. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. All the extracts of *S. rebuadiana* were treated in the similar manner, absorbance was recorded and the percentage of inhibition was calculated according to the following equation:

$$\%\text{inhibition} = \frac{A_0 - A_t}{A_0} \times 100.$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the samples of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

3. Results and discussion

3.1. Total phenolic content

There are increasing evidences that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is a great interest in the protective biochemical functions of natural antioxidants present in spices, herbs and medicinal plants (Gyamfi et al., 2002). The total amount of phenolic content present in the aqueous leaf extract of *S. rebaudiana* (ALES) is shown in Fig. 1. In one gram of aqueous leaf extract, 56.74 mg gallic acid equivalent of phenols was detected while ethanolic leaf extract of *S. rebaudiana* has been reported to show 61.50 mg gallic acid equivalent of phenols (Shukla et al., 2009). These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. From all these observations it can be concluded that the plant extracts with high level of polyphenolic compounds show good antioxidant activity *in vitro* systems. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up

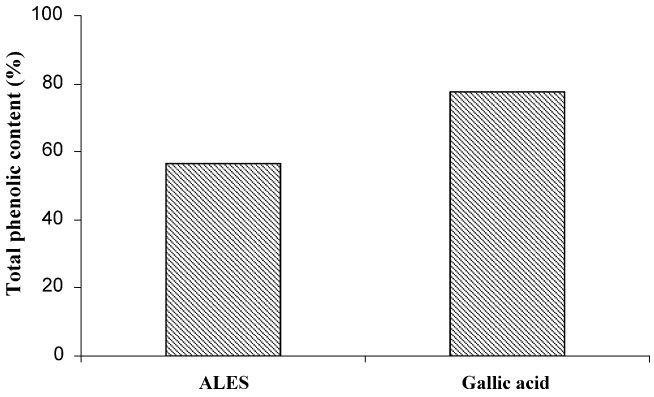

Fig. 1. Amount of total phenolic content in the aqueous leaf extract of *Stevia rebaudiana*. ALES: aqueous leaf extract of *S. rebaudiana*.

to 1 g daily from a diet rich in fruits and vegetables (Tanaka et al., 1998). Methanol extract of *Mucuna pruriens* seeds showed highest total phenolic content and antioxidant activity (Rajeshwar et al., 2005). The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Aneta et al., 2007).

3.2. Inhibition of DPPH radical

DPPH method is widely reported for screening of antioxidants and for determining comparative antioxidant effectiveness (Vani et al., 1997). The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals is initiated by the lipid autoxidation (Ingold et al., 1993). The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Positive DPPH test suggested that the samples were free radical scavengers. On the DPPH radical, aqueous leaf extract had significant radical scavenging effect with increasing concentration in the range of 20–200 μg/ml when compared with that of ascorbic acid, the scavenging effect of aqueous leaf extract was little lower. Similar dose dependent results were observed in methanol extracts of *Camellia sinensis*, *Ficus bengalensis* and *Ficus racemosa* as they contained relatively higher levels of total phenolics then acetone extracts (Manian et al., 2008). A 200 μg/ml of aqueous leaf extract (ALES) and ascorbic acid exhibited 72.37 and 82.58% inhibition, respectively and the IC_{50} values were found to be 83.45 and 26.75 μg/ml for ALES and ascorbic acid, respectively (Table 1). The different concentrations of aqueous leaf extract (20, 40, 50, 100 and 200 μg/ml) showed antioxidant activities in a dose dependent manner (40.00, 46.84, 51.35, 64.26 and 72.37% inhibition), respectively on the DPPH radical scavenging assay (Fig. 2). A higher DPPH radical scavenging activity is associated with a lower IC_{50} value.

3.3. Hydroxyl radical scavenging

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and can cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Manian et al., 2008). Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu et al., 2001). The fentone reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either

Table 1
Effect of aqueous leaf extract of *Stevia rebuadiana* on different radical scavenging activities.

IC50 values of ALES							
DPPH radical scavenging activity	AA	Hydroxyl radical scavenging activity	AA	Nitric oxide radical scavenging activity	AA	Super oxide radical scavenging activity	AA
83.45	26.75	100.86	71.41	98.73	66.01	100.86	36.69

ALES: aqueous leaf extract of *Stevia rebuadiana*; AA: ascorbic acid.

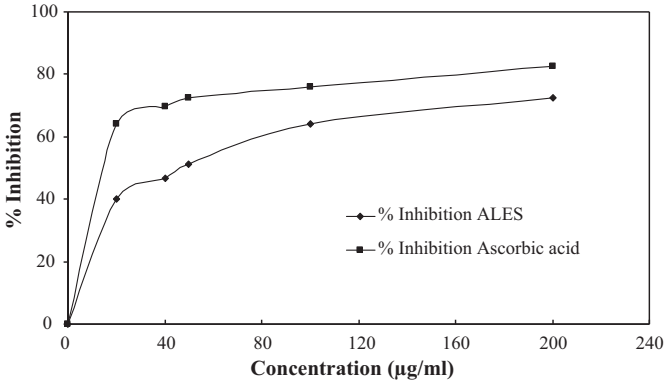

Fig. 2. DPPH radical scavenging activity of the aqueous leaf extract of *S. rebaudiana*. ALES: aqueous leaf extract of *S. rebaudiana*.

at the sugar or the base, giving rise to a large number of products (Rajeshwar et al., 2005). The percent inhibition of ALES on hydroxyl radical scavenging at the used concentrations of 20, 40, 50, 100 and 200 µg/ml was found to be 40.67, 43.11, 53.82, 68.19 and 79.81%, respectively. All results showed antioxidant activity in dose dependent manner. A 200 µg/ml of ALES and ascorbic acid exhibited 79.81 and 72.47% inhibition, respectively (Fig. 3) and their respective IC_{50} values were found to be 100.86 and 71.41 µg/ml (Table 1). The ability of the above mentioned extract to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of the chain reaction. Cox et al. (2005) also reported that the water extracts of *Smilax glyciphylla* also inhibited deoxyribose degradation. Hagerman et al. (1998) explained that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by phenolics than their specific functional groups.

3.4. Nitric oxide radical scavenging

Nitric oxide is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodilatation and control of blood pressure (Jagetia et al., 2002). Nitric oxide plays an important role in various types of inflammatory processes in the animal body. In the present study, the aqueous leaf extract of *S. rebaudiana* (ALES) was checked for its inhibitory effect on nitric oxide production. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by ALES. The various concentrations of ALES (20, 40, 50, 100 and 200 µg/ml) showed (38.56, 42.82, 45.29, 57.39 and 68.38% inhibition), respectively. Results showed the percentage of inhibition was in a dose dependent manner (Fig. 4). Similar findings were observed in case of *M. pruriens* seeds (Rajeshwar et al., 2005). A 200 µg/ml of ALES and ascorbic acid exhibited 68.38 and 71.52% inhibition, respectively. Sreevidya et al. (2006) reported the nitric oxide scavenging activity in hexane, ethylacetate and 80% aqueous alcohol extract of *Chlorophytum tuberosum*, presence of sugars, saponins and tannins indicate the potent antioxidant activity. The concentration of ALES needed for 50% inhibition (IC_{50}) was found to be 98.73 µg/ml, whereas 66.01 µg/ml was needed for ascorbic acid (Table 1). The results were found to be statistically significant ($P < 0.05$).

3.5. Super oxide radical scavenging

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases (Halliwell and Gutteridge, 1999). Different concentrations of ALES (20, 40, 50, 100 and 200 µg/ml) had strong superoxide scavenging activity (27.56, 31.11, 41.68, 64.00 and 72.20% inhibition), respectively. A 200 µg/ml of ALES and ascorbic acid exhibited 72.20 and 85.42% inhibition, respectively (Fig. 5). IC_{50} value of ALES on superoxide radical scavenging activity was found to be 100.86 µg/ml, whereas the IC_{50} value of ascorbic acid was found to be 36.69 µg/ml (Table 1). The aqueous leaf extract of *S. rebaudiana* (ALES) had a scavenging

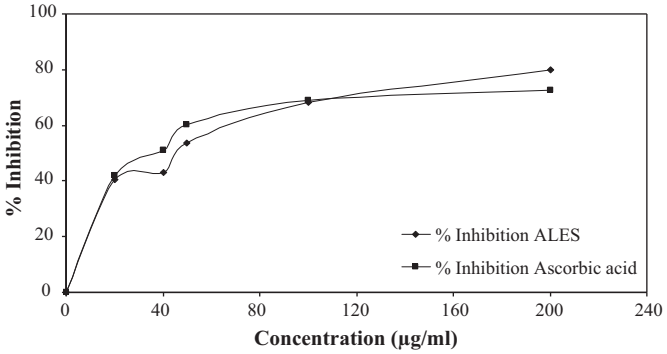

Fig. 3. Hydroxyl radical scavenging activity of the aqueous leaf extract of *S. rebaudiana*. ALES: aqueous leaf extract of *S. rebaudiana*.

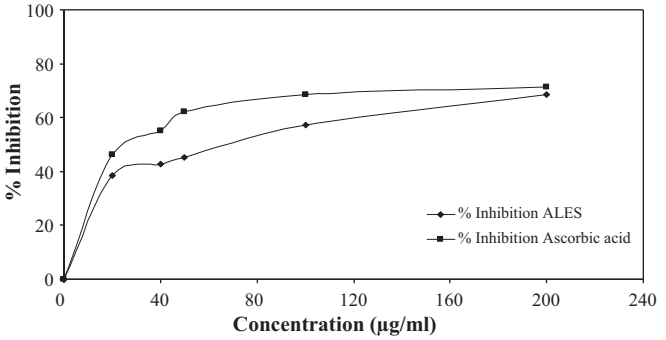

Fig. 4. Nitric oxide radical scavenging activity of the aqueous leaf extract of *S. rebaudiana*. ALES: aqueous leaf extract of *S. rebaudiana*.

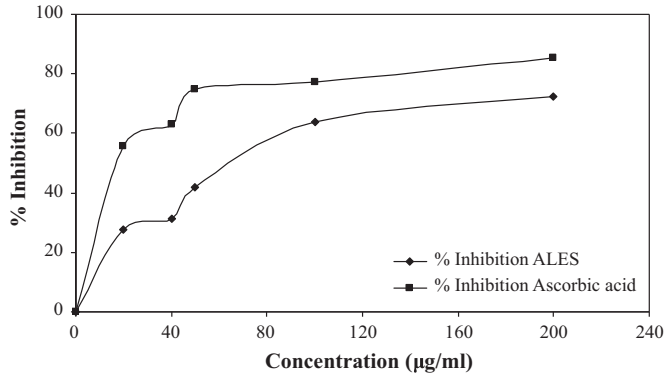

Fig. 5. Super oxide radical scavenging activity of the aqueous leaf extract of *S. rebaudiana*. ALES: aqueous leaf extract of *S. rebaudiana*.

activity on the superoxide radicals in a dose dependent manner (20–200 µg/ml) in the reaction mixture. Nonetheless, when compared to ascorbic acid, the superoxide scavenging activity of the aqueous leaf extract was found to be low. This could be due to the presence of reactive concentration of bioactive constituents and mixture of other nutrients in the extract. Our results showed close agreement with antioxidant activity of alcoholic extract of *Paullinia cupana* (Mattei et al., 1998). Cox et al. (2005) reported that the aqueous extract of *S. glyciphylla* (leaves) and methanolic extract of *Smilax excelsa* (leaves) quenched chemically generated superoxide anion (Ozsoy et al., 2008). Superoxide scavengers and their capacity to scavenge superoxide may contribute to their antioxidant activity. Results were found statistically significant ($P < 0.05$). However a large number of phytocompound groups are implicated for antioxidant activity (Devasagayam et al., 2002). Many authors have also correlated antioxidant activity of various plant species with their polyphenolic or phenolic contents (Kaur and Kapoor, 2002).

In conclusion, this study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. In the present investigation, we demonstrated that aqueous leaf extract of *S. rebaudiana* contained higher levels of total phenolic compounds and was capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as a reducing agent. Significant antioxidant activity of aqueous leaf extract of *S. rebaudiana* provides a scientific validation for the traditional use of this plant as an accessible source of natural antioxidants with consequent health benefits. Further work on isolation and identification of active compounds and their efficacy needs to be done.

Acknowledgements

Authors are grateful to Head Department of Botany and Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar, MP, India, for providing laboratory facilities and Madhya Pradesh Science and Technology, Bhopal, MP, India.

References

Aneta W, Jan O, Renata C. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 2007;105:940–9.
Babu BH, Shylesh BS, Padikkala J. Antioxidant and hepatoprotective effect of *Alanthus icicifocus*. Fitoterapia 2001;72:272–7.
Blios MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;26:1199–200.
Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu OI. In vitro antioxidant properties of dantrolene sodium. Pharmacol Res 2001;44:491–4.
Chan P, Linson B, Chen Y, Liu J, Hsieh M, Cheng J. A double blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br J Clin Pharmacol 2000;50:215–20.
Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agric 2000;80:561–6.
Cox SD, Jayasinghe KC, Markham JL. Antioxidant activity in Australian native sarsaparilla (*Smilax glyciphylla*). J Ethnopharmacol 2005;101:162–8.
Devasagayam TPA, Tilak JC, Baloor KK, Sane KS, Ghaskadbi SS, Lele RD. A review: free radicals and antioxidants in human health. Curr Stat Fvt Prosp JAPI 2002;52:794–804.
Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi S, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India 2004:794–804.
Dursum E, Otles S, Akcicek E. Herbs as food source in Turkey. Asian Pacific J Cancer Prev 2004;5:334–9.
Farber JL. Mechanisms of cell injury by activated oxygen. Environ Health Perspect 1994;102:17–24.
Geuns JC. Safety of stevia and stevioside. Recent Res Dev Phytochem 2000;4:75–88.
Goenadi DH. Water tension and fertilization of *Stevia rebaudiana* Bertoni on toxic Tropudalf. Indian J Pharmacol 1983;51:85–90.
Gyamfi MA, Yonamine M, Aniya Y. Free radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally induced liver injuries. Gen Pharmacol 2002;32:661–7.
Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887–92.
Halliwell B, Gutteridge JMC, Amoma OL. The deoxyribose method: a simple test tube assay for the determination of rate constant for reaction of hydroxyl radical. Anal Biochem 1987;165:215–9.
Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 1999.
Hochestein P, Atallah AS. The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. Mut Res 1988;202:363–75.
Ingold KU, Bowry VW, Stocker R, Walling C. Autoxidation of lipids and antioxidation by alpha-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: unrecognized consequences of lipid particle size as exemplified by oxidation of human low density lipoprotein. Proc Natl Acad Sci USA 1993;90:45–9.
Jagetia GC, Baliga MS, Malagi KJ, Kamath MS. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to γ-radiation. Phytomedicine 2002;9:99–108.
Jutabha PC, Chatsudthipong V. Effect of stevioside on PAH transport by isolated perfused rabbit renal proximal tubule. Can J Physiol Pharmacol 2000;78:737–44.
Kaur C, Kapoor HC. Antioxidant activity and phenolic content of some Asian vegetables. Int J Food Sci Technol 2002;37:153–61.
Larson RA. The antioxidants of higher plants. Phytochemistry 1988;27(4):969–78.
Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. Food Chem 2008;107:1000–7.
Marcocci L, Maguire JJ, Droy MT. The nitric oxide scavenging properties of Gingo biloba extract EGb 761. Biochem Biophysics Res Commun 1994;15:748–55.
Mattei R, Dias RF, Espinola FB, Carlini EA, Barros SBM. Guarana (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity in vitro. J Ethnopharmacol 1998;60:111–6.
Melis MS. Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: renal effects. J Ethnopharmacol 1995;47:129–34.
Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excelsa* leaf extracts. Food Chem 2008;110:571–83.
Rajeshwar Y, Kumar GP, Gupta M, Mazumder UK. Studies on In vitro antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. Eur Bull Drug Res 2005;13:31–9.
Sabu MC, Ramadasan K. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol 2002;81:155–60.
Schuler P. Natural antioxidants exploited commercially. In: Hudson BJF, editor. Food antioxidants. London: Elsevier; 1990. p. 99–170.
Shukla S, Mehta A, Bajpai VK, Shukla S. In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food Chem Toxicol 2009;47:2338–43.
Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. Am J Enol Viticult 1977;28:49–55.
Sreevidya N, Govindrajan R, Vijayakumar M, Mehrotra S. Free radical scavenging potential of *Chlorophytum tuberosum* Baker. J Ethnopharmacol 2006;104:423–5.
Suanarunsawat T, Chaiyabutr N. The effect of stevioside on glucose metabolism in rat. Can J Physiol Pharmacol 1997;75:976–82.
Tanaka M, Kuei CW, Nagashima Y. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi 1998;47:1409–14.
Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Int J Pharmacog 1997;35:313–7.