Indian Journal of Biochemistry & Biophysics
Vol. 46, February 2009, pp. 122-125

SHORT COMMUNICATION

Antioxidant potential of the root of *Vetiveria zizanioides* (L.) Nash[#]

Suaib Luqman*, Ritesh Kumar, Shubhangi Kaushik, Suchita Srivastava, Mahendra P Darokar and Suman P S Khanuja

Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (Council of Scientific and Industrial Research), P.O. CIMAP, Lucknow 226 015, India

Received 17 July 2008; revised 24 December 2008

Vetiveria zizanioides, an aromatic plant commonly known as vetiver has been used for various ailments. The essential oil of vetiver root has been shown to possess antioxidant activity. However, antioxidant potential of spent root extract has not been reported. Hence, in the present study, ferric reducing, free radical scavenging and antioxidant activity of two genotypes namely KS1 and gulabi of *V. zizanioides* L. Nash root were investigated using *in vitro* assays — the ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), total phenolic content (TPC), total antioxidant capacity (TAC) and reducing power (RP). KS1 genotype showed higher FRAP values, DPPH inhibition, TPC and RP potential compared to gulabi and the antioxidant activity increased with the concentration of the extract (10-1000 µg/mL). A significant protective effect of cv KS1 (100 µg/mL) extract was also observed in reduced glutathione (GSH) and malondialdehyde (MDA) concentrations of erythrocytes subjected to oxidative stress by tert-butyl hydroperoxide (*t*-BHP) and hydrogen peroxide (H_2O_2). The cv KS1 showed better antioxidant activity, compared to cv gulabi indicating the possibility of exploring the presence of different phytoconstituents in the two varieties.

Keywords: *Vetiveria zizanioides*, Antioxidant, Erythrocytes, Glutathione, Malondialdehyde

*Author for correspondence
Ph: +91-522-2717529; Fax: +91-522-2342666
E-mail: s.luqman@cimap.res.in

Abbreviations: CAT, catalase; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; FRSA, free radical scavenging activity; GAE, gallic acid equivalents; GD, gulabi spent (distilled) root extract; GU, gulabi intact (undistilled) root extract; GSH, reduced glutathione; KSD, KS1 spent (distilled) root extract; KSU, KS1 intact (undistilled) root extract; MDA, malondialdehyde; POD, peroxidase; RP, reducing power; SOD, superoxide dismutase; TAC, total antioxidant capacity; *t*-BHP, tert-butyl hydroperoxide; TCA, trichloroacetic acid; TPC, total phenolic content

[#] CIMAP Communication No. 2008-10A

Vetiveria zizanioides, popularly known as 'Khus' grass is the major source of well-known oil of vetiver, which is used in medicine and in perfumery[1]. Different parts of plant including roots are used for the treatment of ailments, such as mouth ulcer, fever, boil, epilepsy, burn, snake, scorpion bite, rheumatism, headache, weakness, toothache, sprain, malaria, acidity relief, urinary tract infection and against various fungal and bacterial diseases[2]. *V. zizanioides* is considered useful in the rehabilitation of metalliferous mine wastelands, as the presence of the Pb and Zn greatly enhances the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)[3] implying different mechanisms to detoxify active oxygen species exist in different parts of the plants[4].

Recently, antioxidant activity in vetiver oil has been attributed to β-vetinene, β-vetinone and α-vetinone[5]. However, ferric reducing, free radical scavenging and antioxidant potential of vetiver root (intact and spent) extract have not been reported. Therefore, in the present study, the concentration-dependent ferric reducing, free radical scavenging and antioxidant potential of root extract of two genotypes of vetiver have been investigated. The protective effect of vetiver root extract on lipid peroxidation and reduced glutathione concentration have also been evaluated in oxidatively stressed erythrocytes.

Materials and Methods

Collection of plant material

CIMAP has a large collection of germplasm of vetiver grass from different parts of India and the roots of two genotypes (gulabi and KS1) of *V. zizanioides* L. Nash (Poaceae) were collected at similar developmental stage and in the same season from the research farm. The herbarium specimens were deposited (CIMAP-8897 for KS1 and CIMAP-8898 for gulabi) at Gyan Surabhi of our Institute.

Extract preparation and assay of antioxidant parameters

Hexane extract of the spent and intact roots of two vetiver cultivars were prepared[2] and dissolved in dimethyl sulfoxide (DMSO) with a final

concentration of 0.01%. Ferric reducing antioxidant power (FRAP) assay was carried out by the method as described previously[6,7]. The 1-1diphenyl-2-picrylhdrazyl (DPPH) radical scavenging activity of extract was measured as described previously[8]. The total phenolic content of extracts was determined in terms of gallic acid equivalence by Folin-Ciocalteu reagent method[9]. The total antioxidant capacity (TAC) of extracts was measured using ascorbic acid as the standard[10]. The reducing power (RP) of extracts[11], reduced glutathione (GSH) concentration[12,13] and erythrocyte malondialdehyde (MDA) formed during lipid peroxidation were also measured[14,15].

Statistical analysis

The results were given as mean ± SD of three independent experiments in replicate. The data were analyzed using student's *t* test to make a statistical comparison between two-tailed paired groups. A comparison was done with the control *vs.* oxidized group and oxidized *vs.* extract-treated group. The significance levels were set at $p < 0.001$, < 0.01 and/or < 0.05. Correlation among the antioxidant parameters was done by Pearson's co-efficient.

Results and Discussion

A concentration-dependent ferric reducing, free radical scavenging and total antioxidant potential of hexane extracts of root (intact and spent) were evaluated in two genotypes of *V. zizanioides*. Activity of extracts were tested by performing *in vitro* FRAP, DPPH, TPC, RP and TAC assay and observations are presented in Fig. 1 A-E. The antioxidant activity increased with the increase in concentration of the extract. FRAP expressed in equivalence of ferrous sulphate (μM/L), increased with the increasing concentration of the extract (Fig. 1A). KSD (spent root extract of KS1) showed higher FRAP than the other three extracts (KSD>GD>KSU>GU) at a final concentration of 1 mg/mL. The extracts also reduced

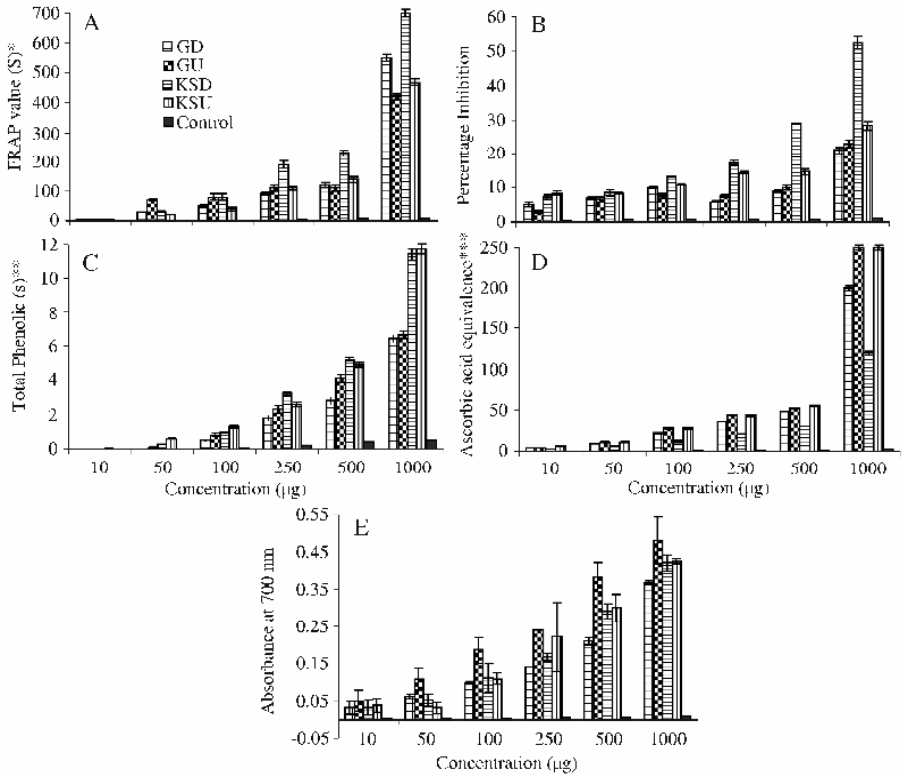

Fig. 1—Concentration-dependent ferric reducing antioxidant power (**A**), free radical scavenging activity (**B**), total phenolic content (**C**), total antioxidant capacity (**D**) and reducing power (**E**) of (intact and spent) root extracts of two genotypes of *V. zizanioides*. [*FRAP values expressed as μ*M* of ferrous sulphate equivalence, **Total phenolic content expressed in terms of μg gallic acid equivalence, ***Total antioxidant capacity expressed in terms of μg ascorbic acid equivalence. Values are mean ± SD of three independent experiments in replicates at each concentration. GD, gulabi spent (distilled) root extract; GU, gulabi intact (undistilled) root extract; KSD, KS1 spent (distilled) root extract; KSU, KS1 intact (undistilled) root extract]

Table 1—Protective effect of vetiver root extracts (100 µg/mL) on reduced glutathione (GSH) and malondialdehyde (MDA) concentration of erythrocytes stressed by H_2O_2 and *t*-BHP

[Values are mean ± SD of three independent experiments in replicate at each concentration]

Erythrocytes stressed by H_2O_2	Control	H_2O_2	Quercetin (10 µg/mL)	GD	GU	KSD	KU
GSH	2.17 ± 0.62	0.915 ± 0.16[a]	1.266 ± 0.13	2.187 ± 0.59[b]	2.18 ± 0.82[b]	2.03 ± 0.77[c]	2.21 ± 0.77[b]
MDA	0.049 ± 0.005	0.105 ± 0.028[b]	0.0406 ± 0.004[b]	0.042 ± 0.03[b]	0.043 ± 0.04[b]	0.045 ± 0.027[b]	0.063 ± 0.026[b]
Erythrocytes stressed by t-BHP	Control	*t*-BHP	Quercetin (10 µg/mL)	GD	GU	KSD	KU
GSH	1.99 ± 0.28	1.03 ± 0.67[b]	1.19 ± 0.52	0.041 ± 0.25	1.07 ± 0.75	0.191 ± 0.1	0.122 ± 0.27
MDA	0.049 ± 0.005	0.086 ± 0.014[c]	0.0376 ± 0.005[b]	0.135 ± 0.08	0.133 ± 0.12	0.028 ± 0.03[b]	0.045 ± 0.01[c]

a: $p < 0.001$, b: $p < 0.01$, c: $p < 0.05$

GD, gulabi spent (distilled) root extract; GU, gulabi intact (undistilled) root extract; KSD, KS1 spent (distilled) root extract; KSU, KS1 intact (undistilled) root extract; Concentration of GSH and MDA is expressed as µmol/ml erythrocytes and µmol/ml of packed erythrocytes, respectively

the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. KSD was a better scavenger as compared to other three extracts (KSD>KSU>GU = GD; Fig. 1B). A Pearson coefficient of 0.8919 was observed, showing a positive correlation between DPPH and FRAP.

As plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, their total amount in the root extracts was determined. The content of phenolics was highest in KSD and lowest in GD (spent root extract of gulabi) and the phenolic content followed the order: KSD = KSU>GU = GD at the final concentration of 1 mg/mL (Fig. 1C). A strong positive correlation was also observed between TPC and FRAP (Pearson coefficient 0.924) and TPC and DPPH (Pearson coefficient 0.879).

The total antioxidant capacity was found in the following order: KSU = GU>GD>KSD at a final concentration of 1 mg/mL. The correlation between the total phenolics and total antioxidant capacity was examined as some reports suggested that high TPC increases the antioxidant activity and there is a linear correlation between total phenolic content and antioxidant activity[16,17]. The correlation between TAC and FRAP, DPPH, and TPC was found to be 0.833, 0.597, 0.824, respectively. Although KSD was found to possess high FRAP, DPPH and TPC activity, it showed low TAC values (Fig. 1D), which might be due to the loss of ascorbic acid content of KSD during the distillation process. The reducing power found in the following order: GU>KSD = KSU>GD at a final concentration of 1 mg/mL (Fig. 1E). The observed correlation of RP with FRAP, DPPH, TPC, and TAC was 0.835, 0.716, 0.893, 0.832, respectively.

Further to test the efficacy of the root extracts in protection against the oxidative stress, biomarkers (GSH and MDA) concentration in erythrocytes stressed by H_2O_2 and *t*-BHP were measured. MDA concentrations increased (110 and 76%) and intracellular GSH decreased (57.8 and 49.4%) in erythrocytes oxidatively stressed by H_2O_2 and *t*-BHP, respectively. Alteration in the normal level of MDA and GSH concentration in stressed erythrocytes are indicators of an increased pro-oxidant/antioxidant ratio, compared with normal erythrocytes. Our results were in concomitant with those of previous reports on GSH and MDA concentration in erythrocytes[18-20]. The protective effect of root extracts on GSH and MDA concentrations was significant at 100 µg/mL, when stressed by H_2O_2 (Table 1), while in case of the stress induced by *t*-BHP, none of the extracts showed protective effect on GSH concentration. *t*-BHP induces the oxidative stress by forming peroxynitrile radical which is a different mechanism to that of H_2O_2-induced oxidation. The enhancement of the GSH oxidation in the presence of the extracts might be explained as prooxidant activity (Table 1). The lower concentrations of extracts did not show significant protection of GSH and MDA

concentration in erythrocytes against the stress induced by H_2O_2 and *t*-BHP (data not shown).

Based on above observations, we hypothesize that extracts from the spent/waste part may find use as dietary/supplementary antioxidant in nutraceutical and/or cosmoceutical for protection against complications arising from the oxidative stress. The plants produce many types of scavenger molecules, mainly phenolic compounds[21]; two new flavonoids from *V. zizanioides* and *V. nigritana* have recently been reported[22]. The present study emphasizes the importance of waste material from vetiver plant as a potent source of antioxidant and could be explored for isolation of the active principle for commercial utilization.

Acknowledgements

The work was supported by Department of Science and Technology, Ministry of Science and Technology, Government of India under DST-Fast Track Scheme for Young Scientist. Authors are also grateful to Dr. R K Lal, Dr. J R Bahl and Jeevanika for their cooperation.

References

1 Anon (1976) Vetiver In: The Wealth of India, Vol. X, pp 451-457, Publications & Information Directorate, Council of Scientific and Industrial Research, New Delhi, India

2 Luqman S, Srivastava S, Darokar M P & Khanuja S P S (2005) *Pharma Biol* 43, 732-736

3 Han L, Zhang X & Liu B (2005) *Ying Yong Sheng Tai Xue Bao* 16, 2178-2181

4 Pang J, Chan G S, Zhang J, Liang J & Wong M H (2003) *Chemosphere* 52, 1559-1570

5 Kim H J, Chen F, Wang X, Chung H Y& Jin Z (2005) *J Agric Food Chem* 53, 7691-7695

6 Benzie I F F & Strain J J (1996) *Anal Biochem* 239, 70-76

7 Pulido R, Bravo L & Saura-Calixto F (2000) *J Agric Food Chem* 48, 3396-3402

8 Chung Y C, Chang C T, Chao W W, Lin C F & Chou S T (2002) *J Agric Food Chem* 50, 2454-2458

9 Singleton V L & Rossi J A (1965) *Am J Enol Viticul* 16, 144-158

10 Preito P, Pineda M & Aguilar M (1999) *Anal Biochem* 269, 337-341

11 Yen G C & Chen H Y (1995) *J Agric Food Chem* 43, 27-32

12 Beutler E (1971) Reduced glutathione (GSH) In: *Red Cell Metabolism,* pp 103-105,Grunne and Straton, New York

13 Rizvi S I & Luqman S (2002) *Med Chem Res* 11, 301-307

14 Esterbauer H & Cheeseman K H (1990) *Meth Enzymol* 186, 407-413

15 Luqman S & Rizvi S I (2006) *Phytother Res* 20, 303-306

16 Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J & Vavreinova S (2002) *Food Res Int* 35, 207-211

17 Velioglu Y S, Mazza G, Gao L & Oomah B D (1998) *J Agric Food Chem* 46, 4113-4117

18 Reglinski J, Hoey S, Smith W E & Sturrock R D (1988) *J Biol Chem* 263, 12360-12366

19 Bryszewska M, Zavodnik I B, Niekurzale A & Szosland K (1995) *Biochem Mol Biol 37*, 345-354

20 Wang X, Wu Z, Song G, Wang H, Long M & Cai S (1999) *Clin Hemorheol Microcirc* 21, 137-146

21 Rice-Evans C (1995) *Biochem Soc Symp* 61, 103-116

22 Champagnat P, Heitz A, Carnat A, Fraisse D, Carnat A P & Lamaison J L (2008) *Biochem Syst Ecol* 36, 68-70