Concentration-dependent free radical scavenging and ferric reducing ability of *Vetiveria zizanioides* (L.) Nash: Protective effect of vetiver root extract during oxidative stress

Suaib Luqman*, Ritesh Kumar, Shubhangi Kaushik, Suchita Srivastava, Mahendra P Darokar, Suman PS Khanuja.

Genetic Resources and Biotechnology Division

Central Institute of Medicinal and Aromatic Plants (CSIR) P.O. CIMAP Lucknow-226015, India

* Author for Correspondence: s.luqman@cimap.res.in

Financial Support: Department of Science and Technology (DST), Government of India under Fast Track Young Scientist Scheme

Tribes of India use different parts of vetiver for many of their ailments such as mouth ulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting-bite, rheumatism, headache etc

Decoction of root is used for weakness, toothache, root paste for sprain, root vapor for malarial fever, root ash for acidity relief, stem decoction for urinary tract infection and leaf juice as anti-helminthic

Scientific information on antioxidant properties of medicinal plants that is less widely used in culinary and medicine is still rather scarce. Therefore, finding new sources of natural antioxidant remains an interesting and useful task.
Kim et al., (2005) evaluated antioxidant activity in vetiver oil and concluded that β-vetinene, β-vetinone and α-vetinone constituent in the vetiver oil possess strong antioxidant activity
Two new flavonoid from *Vetiveria zizanioides* and *Vetiveria nigritana* has recently been reported by Champagnat et al., (2007).

***Vetiveria zizanioides* (Linn) Nash, Family : Poaceae; Khus oil**

Essential oil used in soap, perfumery, chewing tobacco, pan masala

World demand 250 metric tonnes annually

World production 100 tons annually; India-20 to 25 tons annually

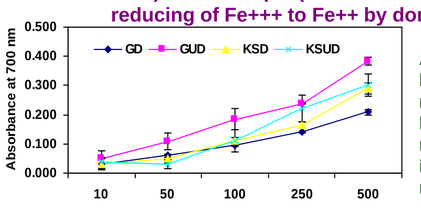
North Indian origin oil is considered to be best in the world

Research Objective (s)

To investigate the concentration-dependent antioxidant potential of two genotypes namely KS1 and gulabi of vetiver roots using *in vitro* assay as described under:

- Reducing power of plants extracts (Yen and Chen, 1995)
- The content of total phenolic compounds in plant extracts by Folin-Ciocalteu reagent method of Singleton and Rossi (1965)
- The total antioxidant capacity of plant extract was measured using the standard method of Preito (1999)
- DPPH radical scavenging activity of plant extract was measured according to method of Chung et al., (2002)
- FRAP assay was carried out by the method of Benzie and Strain (1996) as described by Pulido et al., (2000)
- Hydroxyl radical scavenging activity of two genotypes namely KS 1 and gulabi of vetiver root extract using deoxyribose degradation assay
- Reduced glutathione concentration in erythrocytes was estimated using standard method of Beutler *et al* (1984) as reported by Rizvi and Luqman (2002)
- Erythrocyte malondialdehyde formed during lipid peroxidation was measured according to the method of Esterbauer and Cheeseman (1990) as described previously (2006)

In the reducing power assay, the presence of reductant (i.e. antioxidants) in the sample (extract/antioxidant) would result in the reducing of Fe+++ to Fe++ by donating an electron.

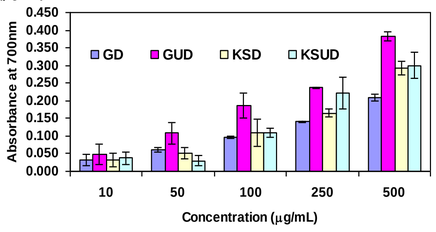


Amount of Fe++ complex can be then be mentioned by measuring the formation of Pearl's Prussian blue at 700 nm. Increase in absorbance indicates an increase in reductive ability

GU>KSD=KSUD>GD

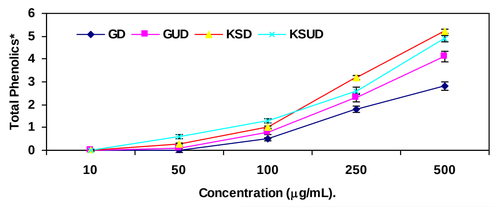
The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.

Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

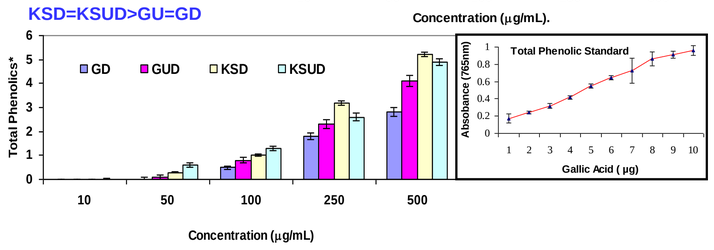


The content of total phenolics in the root extracts of two genotypes of *Vetiveria zizanioides* L. Nash is determined using the Folin–Ciocalteu assay, calculated from standard curve and expressed as gallic acid equivalents (GAE)

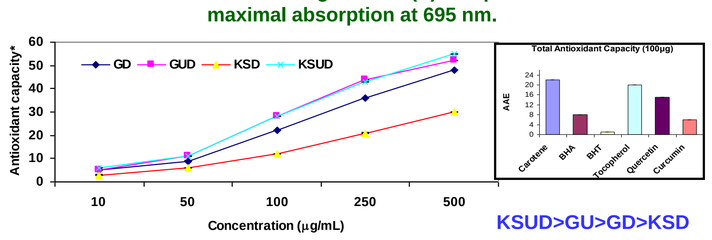
Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it is worth determining their total amount



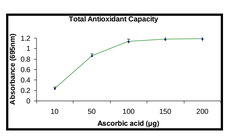
KSD=KSUD>GU=GD

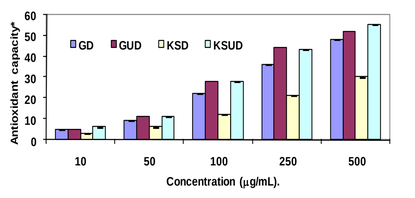


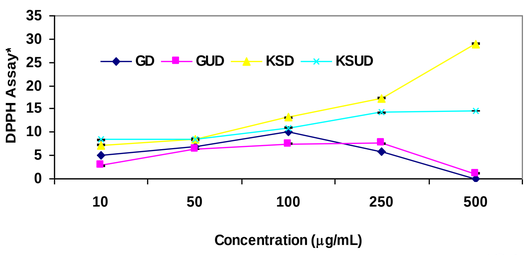
The antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo (VI) to (V) by the antioxidant compounds and the formation of green Mo (V) complex with a maximal absorption at 695 nm.



KSUD>GU>GD>KSD







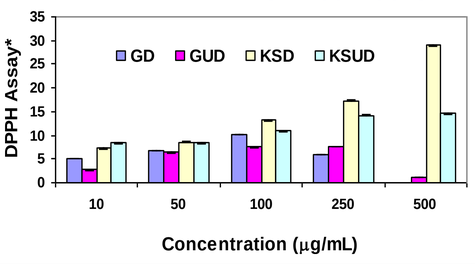
The extracts are able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine.

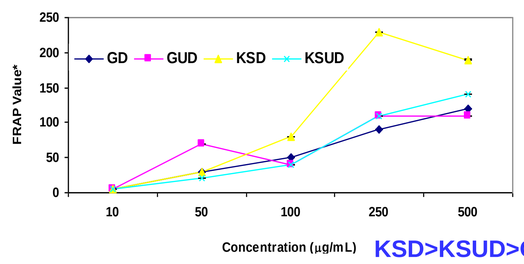
Free radical scavenging activity of antioxidants was evaluated using DPPH as a reagent.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants and plant extracts

KSD>KSUD>GU>GD

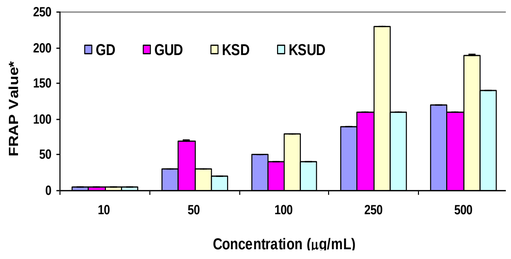


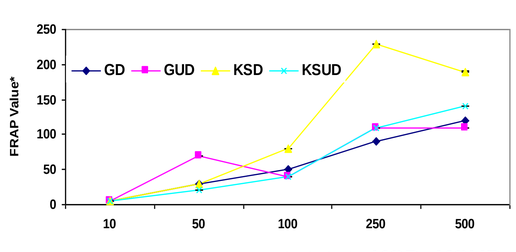


FRAP assay quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidants and plant extracts

KSD>KSUD>GD>GU

FRAP assay measured the change in absorbance at 593 nm owing to the formation of blue color Fe++ from Fe+++ oxidized form by the action of electron donating antioxidants.

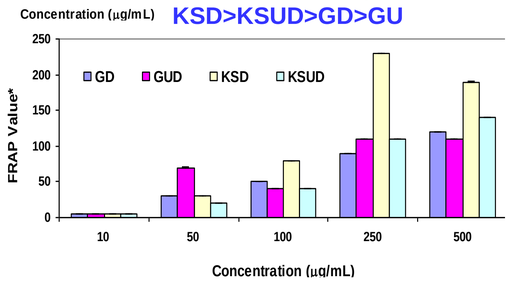




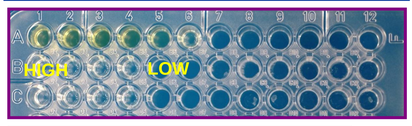
FRAP assay quick and simple to perform, and the reaction is reproducible and linearly related to the molar concentration of the antioxidants and plant extracts

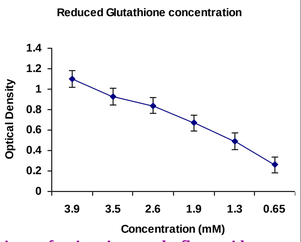
KSD>KSUD>GD>GU

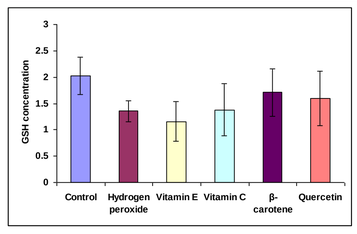
FRAP assay measured the change in absorbance at 593 nm owing to the formation of blue color Fe++ from Fe+++ oxidized form by the action of electron donating antioxidants.



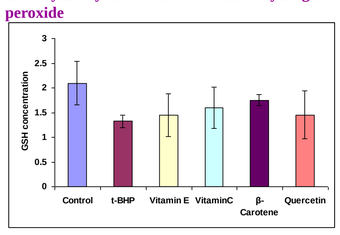
Standard curve of reduced glutathione (GSH)



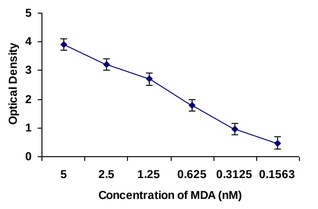




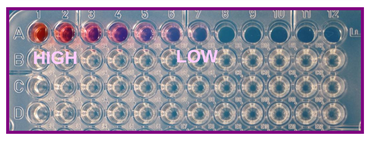
Protection of vitamins and flavonoid on erythrocyte reduced glutathione concentration in erythrocytes stressed with hydrogen peroxide



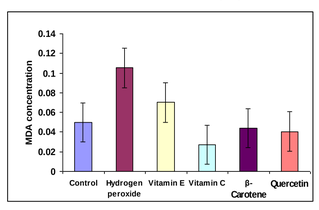
Protection of vitamins and flavonoid on erythrocyte reduced glutathione concentration in erythrocytes stressed with tert butyl hydroperoxide

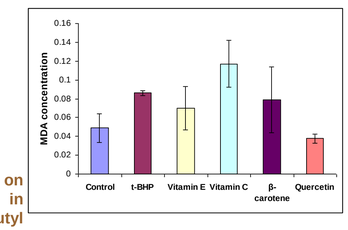


Standard curve of malondialdehyde (MDA)



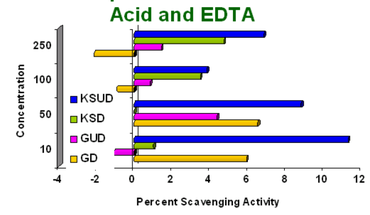
Protection of vitamins and flavonoid on erythrocyte malondialdehyde concentration in erythrocytes stressed with hydrogen peroxide



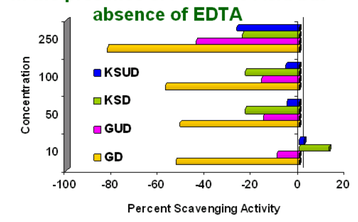


Protection of vitamins and flavonoid on erythrocyte malondialdehyde concentration in erythrocytes stressed with tert butyl hydroperoxide

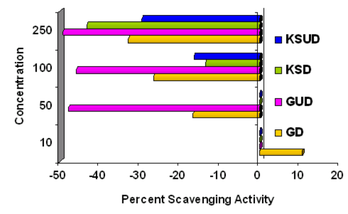
In the presence of Ascorbic Acid and EDTA



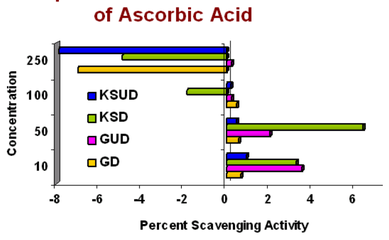
In the presence of Ascorbic Acid but absence of EDTA

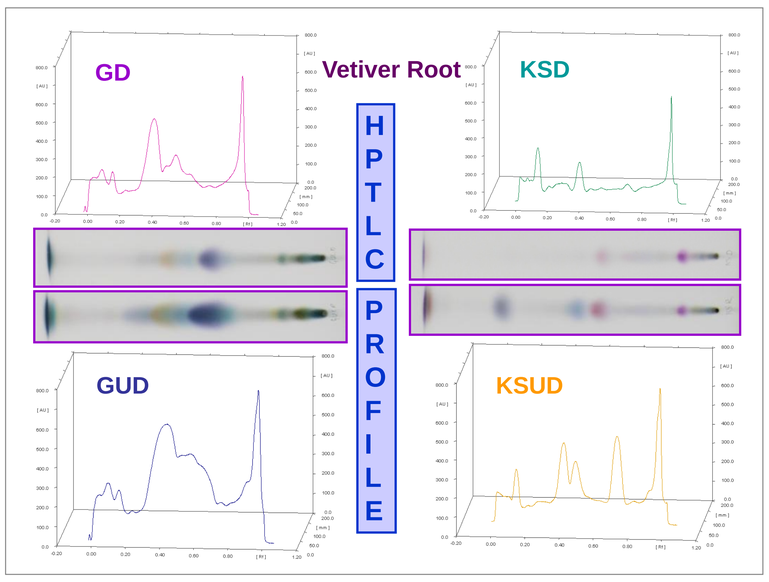


In the absence of both EDTA and Ascorbic Acid



In the presence of EDTA but absence of Ascorbic Acid





CONCLUSION

- Plants, which are more exposed to radical-forming radiation processes, are able to produce many types of scavenger molecules, mainly phenolic compounds.
- Mammals lack the ability to generate phenolic compounds (except oestrogens), but this deficiency may be substituted for, in part, by the plants.
- The useful observation in this study, however, is that the hexane extract of intact as well as spent roots after distilling the oil out showed concentration-dependent ferric reducing antioxidant power and free radical scavenging activity.
- Higher concentration of extract diminishes hydroxyl radical scavenging activity and promotes pro-oxidant activity.
- The present finding has implication of isolating the active molecule useful as dietary/supplementary antioxidant from the waste of vetiver an important plant with high commercial value.
- The genotypic difference observed for level of ferric reducing antioxidant power, free radical scavenging activity being more in one variety (*KS 1*) over other (*gulabi*) indicates the possibility of differences in secondary metabolite formation in *Vetiveria zizanioides*.