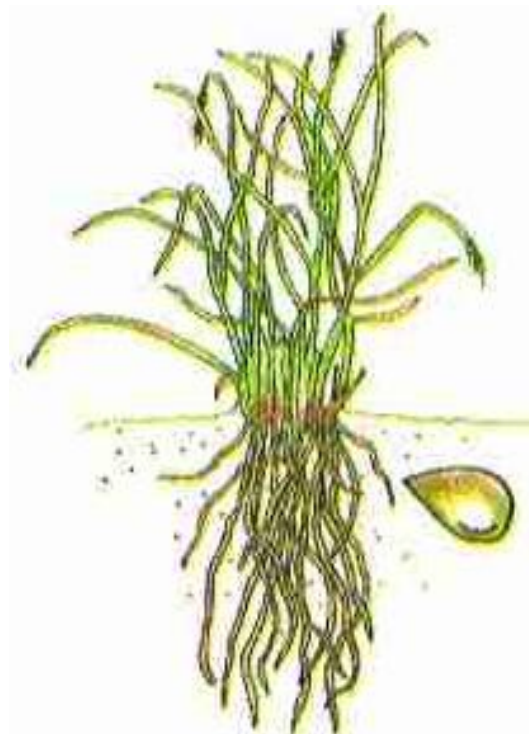


Bioprospecting the efficacy of *Vetiveria zizanioides* L. Nash for novel activities and its safety evaluation



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- ❖ ***Vetiveria zizanioides* L. Nash synonymously known as *Chrysopogon zizanioides* L. Roberty (Family: Poaceae/ Graminae).**
- ❖ **First developed for soil and water conservation by the World Bank during mid 1980s.**
- ❖ **KHUS is the major source of the well-known oil of vetiver, used in medicine, cosmetics, and in perfumery making agarbattis, soaps, soft drinks, pan masala.**
- ❖ **Indian tribes....boils, burns, epilepsy, fever, scorpion sting, snakebite, sores in the mouth, headache, toothache, weakness, lumbago, sprain, rheumatism, urinary tract infection, malarial fever, acidity relief and as an anti-helmintic.**



Uses of Vetiver root and essential oil

Products





- ✿ **Root is important in traditional medicine as a carminative, stimulant and diaphoretic.**
- ✿ **We are studying the biological activity of vetiver root as a part of our effort to discover plant-based biologically active molecules since last many years.**
- ✿ **The plant has not been studied exclusively for detailed pharmacological activities.**
- ✿ **There is a lack of scientific evidence to prove it.**
- ✿ **Hence, there is a need for interaction between scientists, researchers, farmers to find new cultivars/varieties for novel molecules and/or bioactives.**

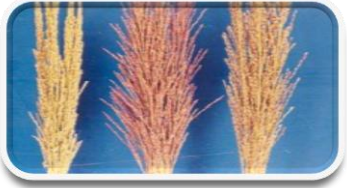
METHODOLOGY & REFERENCES

- **Total Phenolic Estimation:** *American Journal of Enology and Viticulture* (1965); 16: 144-158.
- **Total Antioxidant Capacity Determination:** *Analytical Biochemistry* (1999); 269: 337-341.
- **DPPH Radical Scavenging Activity :** *Journal of Agricultural Food Chemistry* (2002); 50: 2454-2458.
- **Reducing Power Assay:** *Journal of Agricultural Food Chemistry* (1995); 43: 27-32.
- **FRAP Assay:** *Analytical Biochemistry* (1996); 239: 70-76.
- **Hydroxyl Radical Scavenging Assay:** *Biochemical Pharmacology* (2002); 44: 205-214.
- **Glutathione Estimation:** *Pharmaceutical Biology* (2009); 47(6): 483-490.
- **Lipid Peroxidation Estimation:** *Phytotherapy Research* (2006); 20(4): 303-306.
- **Radiometric assay:** *Journal of Clinical Microbiology* (1981); 13: 908-912.
- **Disc Diffusion Assay:** *American Journal of Clinical Pathology* (1966); 36: 493-496.
- **Broth dilution Assay:** *Manual of Clinical Microbiology, 7th ed., Washington DC, The American Society of Microbiology Press, pp. 1526-1543.*

Plant material was collected from the research farm of Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow, India.

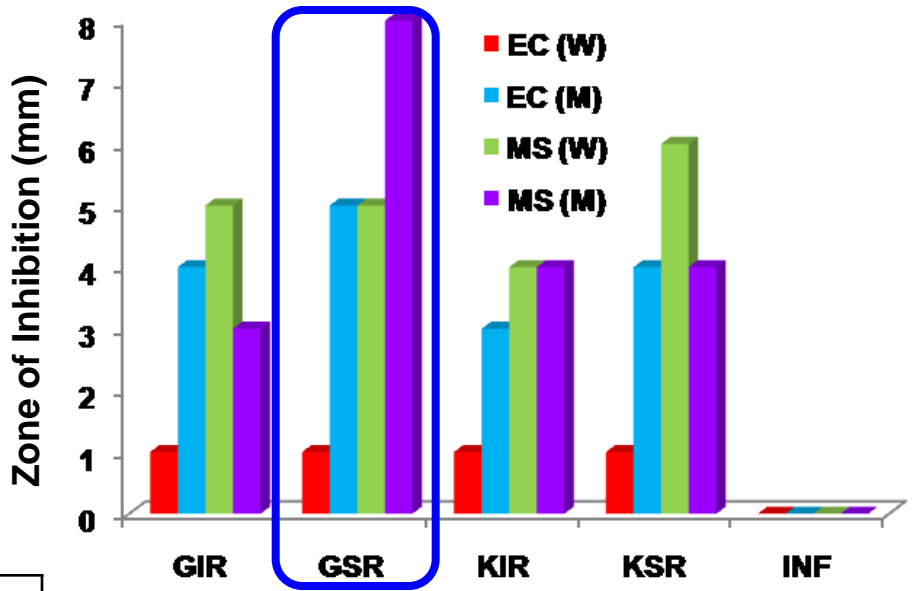
The plant material was authenticated by taxonomists and the voucher specimen (CIMAP herbarium No. 8897 & 8898) was deposited at Gyan Surabhi of CIMAP (CSIR) Lucknow, India.

Detection of antimicrobial activity in spent root of *Vetiveria zizanioides*



- ❖ Essential oil and hexane extract of root was found active against Gram positive bacteria and Yeast.
- ❖ Spent root extract was also found active against drug resistant mutant

MIC ranged from 62.5 to 250 $\mu\text{g/ml}$ (MS)
 MIC ranged from 0.5 to 50 mg/ml (EC)



IC ₅₀	WRL-68 (Liver)	MCF-7 (Breast)	PA-1 (Ovary)	Hepatocytes
GSR	18.75	8.3	5.6	100

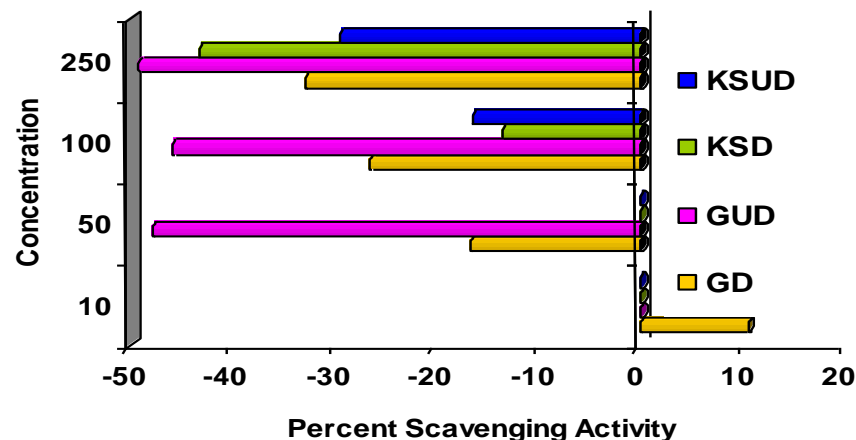
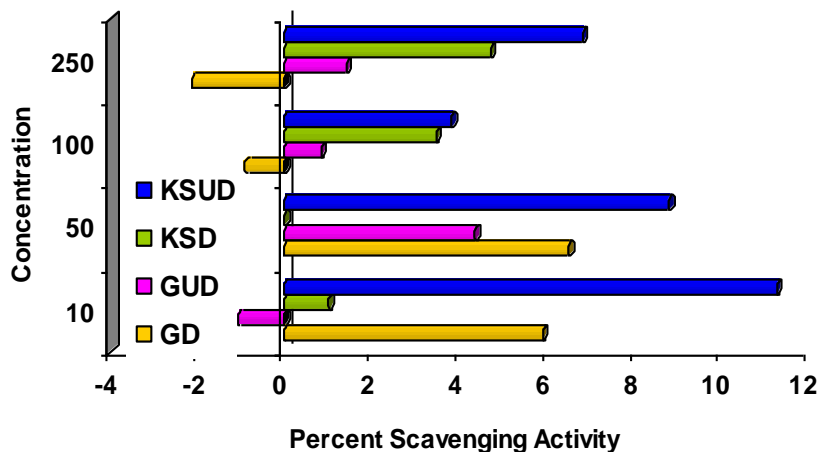
GIR: Gulabi intact root; GSR: Gulabi spent root; KIR: KS 1 intact root ; KSR: KS 1 spent root; INF: Inflorescence
 EC: *Escherichia coli*; MS: *Mycobacterium smegmatis*

Growth Index of *Mycobacterium tuberculosis* H₃₇Rv in presence and/or absence of plant extracts, hexane fraction and antibiotics by BACTEC 460 TB system

Days	Growth Index						
	Ethanolic extract (intact root, 500µg/mL)	Ethanolic extract (spent root, 500µg/mL)	Hexane fraction (50µg/mL)	Rifampicin (0.5µg/mL)	Isoniazid (0.05µg/mL)	Ethambutol (1µg/mL)	Control
1	4	4	4	2	2	4	5
2	7	3	2	2	2	4	8
3	11	2	2	2	2	4	9
4	9	0	0	4	4	5	10
5	14	0	0	3	2	2	16
6	12	0	0	2	0	2	31
7	10	0	0	0	0	2	80

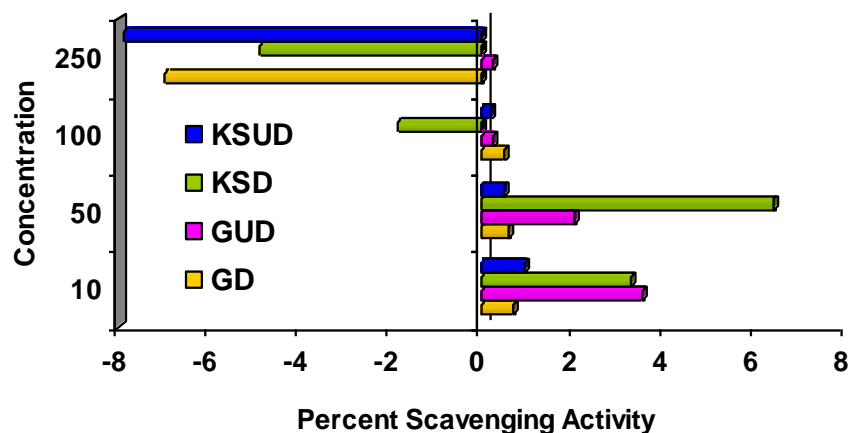
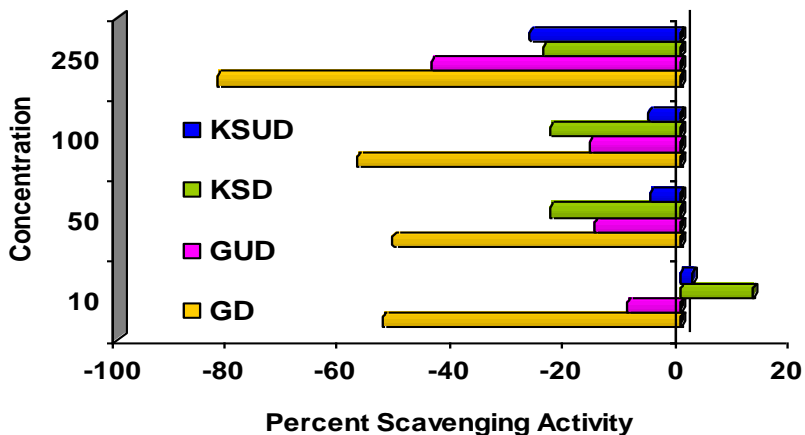
Plant extracts/ antibiotics	Minimal inhibitory concentration (µg/mL)	
	<i>M. tuberculosis</i> H ₃₇ Rv	<i>M. tuberculosis</i> H ₃₇ Ra
Ethanolic extract(intact root)	500	250
Ethanolic extract (spent root)	500	500
Hexane fraction	50	50
Ethyl acetate fraction	NA	NA
Methanol fraction	NA	NA
Rifampicin	0.5	0.5
Isoniazid	0.05	0.05
Streptomycin	2.5	2.5
Ethambutol	1.0	2.5

Hydroxyl radical scavenging activity of root extracts of *Vetiveria zizanioides* L. Nash using deoxyribose degradation assay



Presence of Ascorbic Acid and EDTA

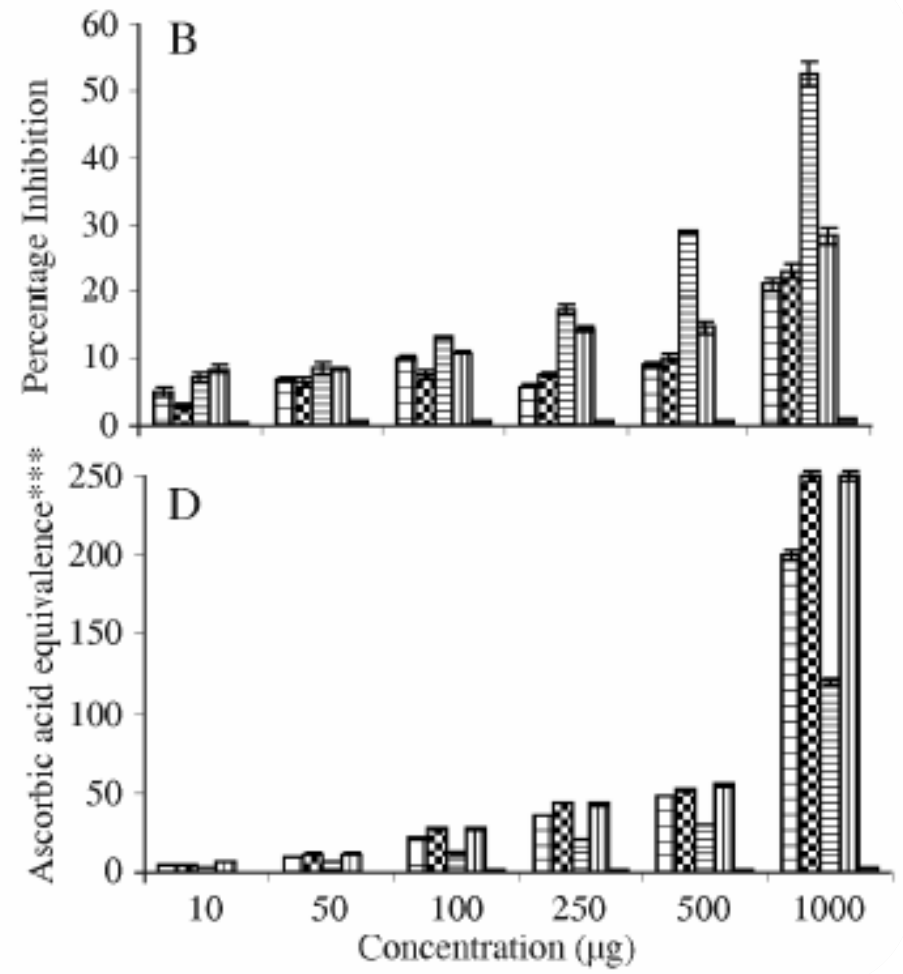
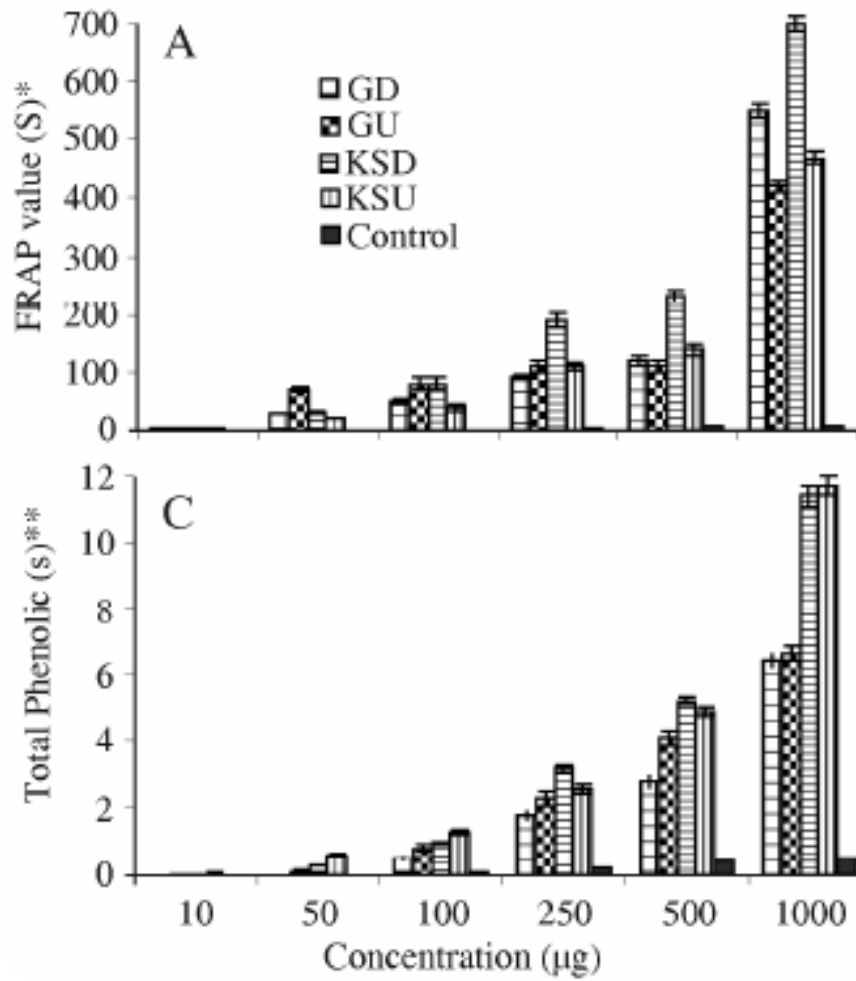
Absence of both EDTA & Ascorbic Acid

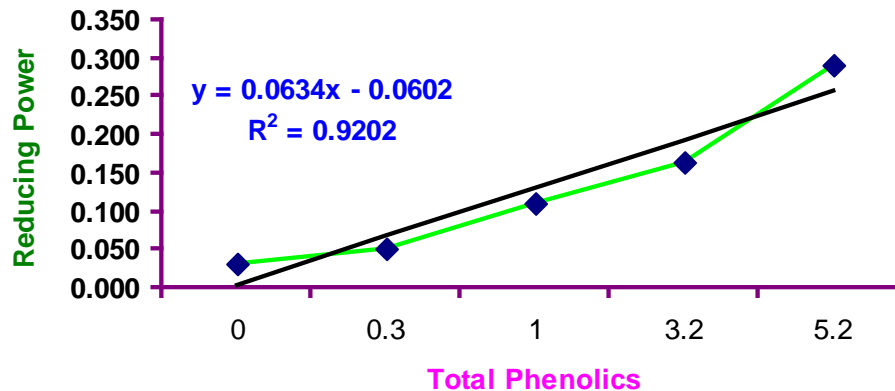
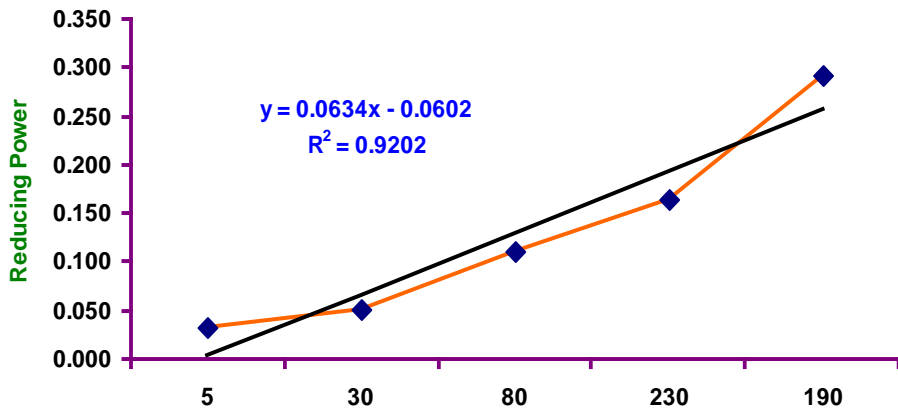
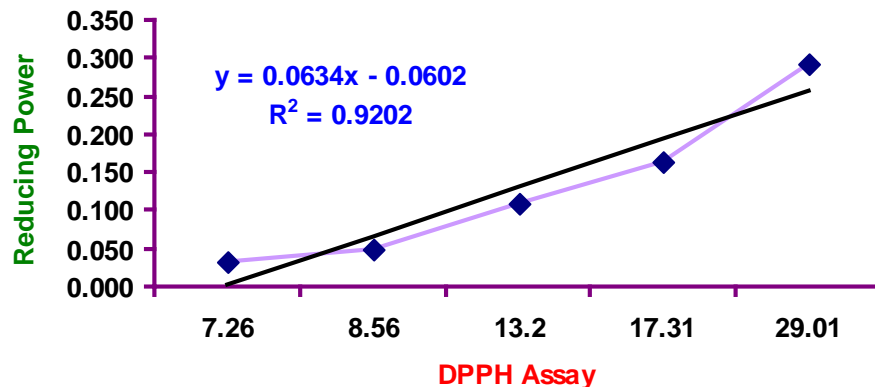
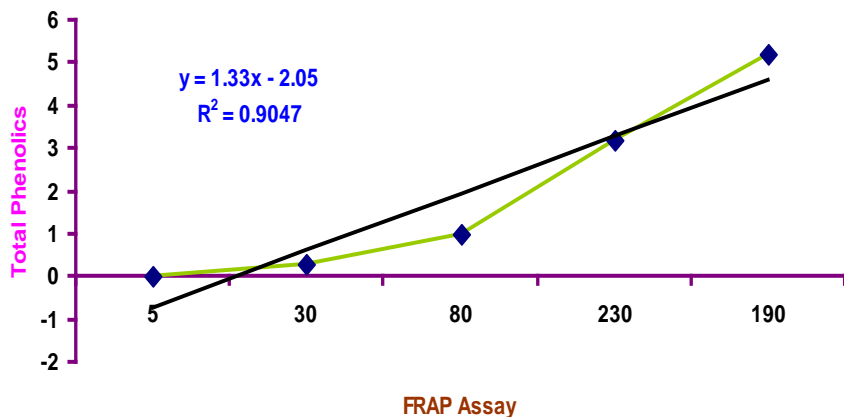
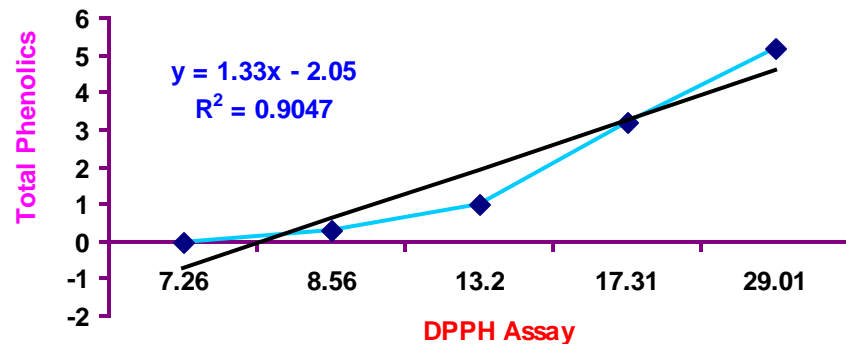
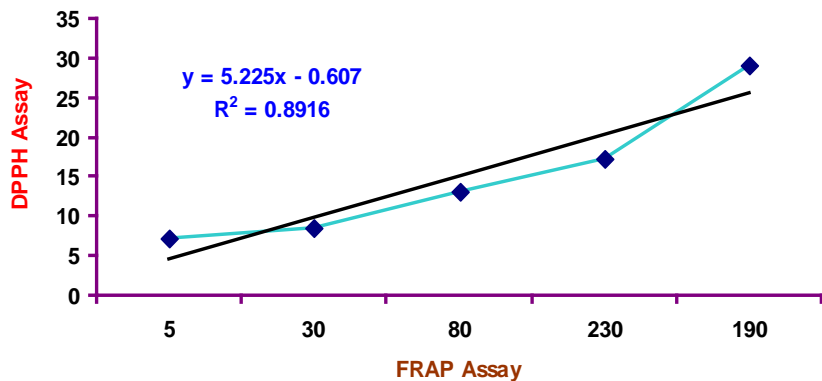


Absence of EDTA

Absence of Ascorbic Acid

Antioxidant property of *Vetiveria zizanioides*





Protective effects of vetiver root extracts (100 µg/mL) on reduced glutathione and malondialdehyde concentration of erythrocytes stressed by hydrogen peroxide

	Control	Hydrogen peroxide	Quercetin (10 µg/mL)	GD	GU	KSD	KSUD
GSH ¹	2.17 ± 0.62	0.915 ± 0.16a	1.266 ± 0.13	2.187 ± 0.59b	2.18 ± 0.82b	2.03 ± 0.77c	2.21 ± 0.77b
MDA ²	0.049±0.005	0.105±0.028b	0.0406±0.004b	0.042± 0.03b	0.043 ± 0.04b	0.045±0.027b	0.063±0.026b

Protective effects of vetiver root extracts (100 µg/mL) on reduced glutathione and malondialdehyde concentration of erythrocytes stressed by tert-butyl hydroperoxide

	Control	Tert-butyl hydroperoxide	Quercetin (10 µg/mL)	GD	GU	KSD	KSUD
GSH ¹	1.99 ± 0.28	1.03 ± 0.67b	1.19 ± 0.52	0.041 ±0.25	1.07 ± 0.75	0.191 ± 0.1	0.122 ±0.27
MDA ²	0.049±0.005	0.086 ± 0.014c	0.0376±0.005b	0.135± 0.08	0.133±0.12	0.028±0.03b	0.045 ±0.01c

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

GD: gulabi spent (distilled) root extract;

KSD: KS 1 spent (distilled) root extract;

Values are mean ± SD of three experiments in replicates at each concentration

GU: gulabi intact (undistilled) root extract;

KSUD: KS 1 intact (undistilled) root extract

Protection Of Vetiver Root Extract During Oxidative Stress

CONCLUSION

- ✓ We showed the potent antibacterial, drug-resistant modifying, hydroxyl radical scavenging, anticancer, and antioxidant activity in intact and spent root of vetiver.
- ✓ The extract showed concentration-dependent ferric reducing antioxidant power and free radical scavenging activity.
- ✓ Higher concentration of the extract diminishes hydroxyl radical scavenging activity and promotes pro-oxidant activity.
- ✓ The genotypic difference observed in one variety (*KS 1*) over other (*gulabi*) indicates the possibility of differences in secondary metabolite formation in *Vetiveria zizanioides*.
- ✓ Our findings support isolation of the active molecule (s) useful as phytoceutical and/or bioactive from vetiver an important plant with high commercial value.
- ✓ Our observation suggests the use of holistic approach for developing potent bioactive (s) with novel mechanism of action.



ACKNOWLEDGEMENT



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DST, NEW DELHI
TEAM CIMAP**



Thank You

PUBLICATIONS

- ❖ **Pharmaceutical Biology 2005, 43: 732-736.**
- ❖ **Journal of Medicinal and Aromatic Plant Sciences 2008, 30: 320-324.**
- ❖ **Indian Journal of Biochemistry and Biophysics 2009, 46: 122-125.**
- ❖ **Complimentary Therapies in Medicine 2011 (Under Review).**

CONFERENCE PAPER

- ❑ **50th Annual Conference of Association of Microbiologists of India at National Chemical Laboratory, Pune, 2009, MM-010, pp 268.**
- ❑ **Society of Free Radical Research-India Satellite Meeting, All India Institute of Medical Sciences, New Delhi, 2008, pp 76.**
- ❑ **National Convention & Seminar on Business enabling of Aromatic Plants and Products, HRDI, Centre for Aromatic Plants, Dehradun, 2007, pp 07.**