Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: www.informahealthcare.com/journals/iphb20

Detection of Antibacterial Activity in Spent Roots of Two Genotypes of Aromatic Grass *Vetiveria zizanioides*.

Suaib Luqman, Suchi Srivastava, Mahendra P. Darokar & Suman P.S. Khanuja

To cite this article: Suaib Luqman, Suchi Srivastava, Mahendra P. Darokar & Suman P.S. Khanuja (2005) Detection of Antibacterial Activity in Spent Roots of Two Genotypes of Aromatic Grass *Vetiveria zizanioides*., Pharmaceutical Biology, 43:8, 732-736, DOI: 10.1080/13880200500387471

To link to this article: https://doi.org/10.1080/13880200500387471

Pharmaceutical Biology
2005, Vol. 43, No. 8, pp. 732–736

Taylor & Francis
Taylor & Francis Group

Detection of Antibacterial Activity in Spent Roots of Two Genotypes of Aromatic Grass *Vetiveria zizanioides*

Suaib Luqman, Suchi Srivastava, Mahendra P. Darokar, and Suman P.S. Khanuja

Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants, Lucknow, India

Abstract

Hexane extracts of inflorescence, intact roots and spent roots, upon hydrodistillation extracting the essential oil from *Vetiveria zizanioides* L. in two genotypes (*gulabi* and KS-1), were evaluated for antibacterial activity against wild-type and drug-resistant strains of *Mycobacterium smegmatis* and *Escherichia coli* using disk diffusion and micro-broth dilution methods. The hexane extracts of intact roots and spent roots after distillation of both varieties were found to show potent activity against the drug-resistant strains of *M. smegmatis* and *E. coli*. The minimum inhibitory concentration of the intact and spent root extracts of both varieties against *M. smegmatis* ranged from 62.5 to 250 μg/ml, and for *E. coli*, the range was from 0.5 mg/ml to 60 mg/ml. Inflorescence in both cases was inactive in terms of antibacterial properties. The results showed that *Vetiveria zizanioides* cv *gulabi* has better inhibitory activity against drug-resistant strains of *M. smegmatis* and *E. coli*, as compared with KS-1. The results suggest DNA gyrase inhibitory action, because the strains resistant to quinolones due to gyrase mutations are sensitive to the extracts.

Keywords: Disk diffusion, *Escherichia coli*, MIC, *Mycobacterium smegmatis*, *Vetiveria zizanioides*.

Introduction

Infectious diseases are the second leading cause of the death worldwide and the third leading cause of death in economically developed countries. Surprisingly, despite increased bacterial resistance to existing drugs, antibiotic development in the pharmaceutical industry is steeply declining (Projan, 2003; Nathan, 2004; Wenzel, 2004).

Quinolones and fluoroquinolones, the synthetic derivatives of nalidixic acid, were first described as antimicrobial agents in 1984 and display broad-spectrum antibacterial activity including anti-mycobacterial activity (Brown & Reeves, 1997; Greenwood, 1997; Watt, 1997). The bactericidal effects of quinolones and fluoroquinolones involve interaction of the drugs with the enzymes called deoxyribonucleic acid gyrase (DNA gyr) and topoisomerase (DNA topo) involved in DNA replication (Pan et al., 1996). Emergence of resistance against quinolone and fluoroquinolone antibiotics is reported to limit their clinical usefulness. Because the mechanism of action of all the quinolones and fluoroquinolones against bacteria is similar, development of resistance to one of the antibiotics would confer simultaneous cross-resistance to other quinolone and fluoroquinolone drugs (Blanchard, 1996). Hence, the hunt is on for the identification of a plant-based biologically active molecule/component that can specifically kill quinolone- and fluoroquinolone-resistant bacteria so that the development of drug resistance in the microbes is not encountered.

Vetiveria zizanioides (L.) Nash (Poaceae), popularly known as *khus grass*, has been known in India since ancient times. It is the major source of the well-known oil of vetiver, which is used in medicine and in perfumery (Rao & Suseela, 2000). In India, the roots have been used for making screens, mats, hand fans, and baskets. Different morphological parts of the grass are used for various ailments, such as boils, burns, epilepsy, fever, scorpion sting, snakebite, and sores in the mouth. The root extract is used for headache and toothache, the leaf paste is used for lumbago, sprain, and rheumatism, the stem decoction for urinary tract infection, the leaf juice as an anthelmintic,

Accepted: September 14, 2005

Address correspondence to: Dr. Suman Preet Singh Khanuja, Director, Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India. Fax: +91-522-2342666; E-mail: khanujazy@yahoo.com

DOI: 10.1080/13880200500387471

the vapors for malarial fever, and the root ash is given for acidity relief (Singh & Maheswari, 1983; Jain, 1991).

In view of the above facts and as a part of our efforts to discover plant-based biologically active molecules/compounds, it was thought worthwhile to study and evaluate the effect of different extracts of the root and inflorescence of *Vetiveria zizanioides* cv *gulabi* and KS-1 against wild-type and drug-resistant strains of *Mycobacterium smegmatis* and *Escherichia coli*.

Materials and Methods

Collection of plant material

The plant materials (root and inflorescence) of *Vetiveria zizanioides* cv *gulabi* and KS-1 were collected from the research farm of Central Institute of Medicinal and Aromatic Plants (CSIR). Drs. J.R. Bahl, R.K. Lal, and S.P. Jain authenticated the plant materials; the voucher specimens were deposited at the CIMAP herbarium (CIMAP-8897 for KS-1 and CIMAP-8898 for *gulabi*), Lucknow, India.

Preparation of extracts

After hydrodistillation of the fresh roots, the spent root and the intact roots were dried under shade at room temperature. After drying, each plant part was chopped into small pieces and then dipped in hexane in a percolator for 24 h at room temperature. Each extract was collected for a week, then filtered using Whatmann filter paper no. 1 and concentrated at 60°C under reduced pressure (335 lb); the so-formed material was finally lyophilized to obtain fine crude extract. The weights of the lyophilized extracts are reported as percentage yield in Table 1. Extracts were stored in a refrigerator until needed for the analysis.

Preparation of the stock solution

Stock solutions of each extract (100 mg/ml) were made in dimethyl sulfoxide (DMSO; Merck Ltd, Mumbai, India Ltd.) and tested for antimicrobial activity against quinolone/fluoroquinolone- and multidrug-resistant mutants of *M. smegmatis* and *E. coli* using disk diffusion and micro-broth dilution assays.

Table 1. Plant parts used for preparing extracts of *Vetiveria zizanioides*.

Plant part	Variety	Extracting solvent	Percentage yield of extracts (%)
Root (intact)	*gulabi*	Hexane	2.50
Root (spent)	*gulabi*	Hexane	1.75
Inflorescence	*gulabi*	Hexane	0.16
Root (intact)	KS-1	Hexane	0.41
Root (spent)	KS-1	Hexane	0.95
Inflorescence	KS-1	Hexane	0.14

Bacterial strains used

Wild-type and drug-resistant strains of *Mycobacterium smegmatis* and *Escherichia coli* were used in the current study (Table 2).

Antibacterial agents used as standard

Ciprofloxacin (25 mg/ml), lomofloxacin (50 mg/ml), and nalidixic acid (50 mg/ml) were used as positive controls, and DMSO was used as a negative control.

Antibacterial activity determination

The antibacterial disk diffusion assay was carried out on Luria agar plates following the method described by Bauer et al. (1966). Bacterial inoculums were prepared from overnight grown cultures (24 h) in Luria broth (Hi Media, Mumbai, India), and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 1.2×10^8 CFU/ml). Aliquots (100 µl) of inoculums were spread over the surface of agar plates with a sterile glass spreader. Five microliters of each extract was put on the paper disk (5-mm diameter, Whatman filter paper no. 3), air-dried, and then placed on the premade bacterial lawns. The plates were then incubated for 16–24 h at 37°C, and the zone of complete growth inhibition was measured. The values are reported as the mean of five experiments in triplicate.

Minimum inhibitory concentration (MIC) determination

The MIC of the hexane extracts of root and inflorescence of *Vetiveria zizanioides* cv *gulabi* and KS-1 against the drug-resistant strains of *M. smegmatis* and *E. coli* were determined by two-fold serial microdilution broth assay using sterile 96-well flat-bottom microtiter plates as described by Jorgenson et al. (1999). The extracts were diluted to final concentrations of 500, 250, 125, 62.50, 31.25, 15.625, and 0.78125 µg/ml. The microtiter plates were inoculated with 10 µl of diluted 24 h grown culture of test organism with a titer of approximately 2×10^4 CFU/ml (equivalent to 0.5 McFarland units). The inoculated microtiter plates were then incubated at 37°C for 16–24 h, and the growth was recorded spectrophotometrically at 600 nm using a Spectramax 190-microplate reader (Molecular Devices, Sunnyvale, CA, USA). The MIC (IC_{80}) value was detected from the turbidimetric data as the lowest concentration of extracts showing growth inhibition equal to or greater than 80% as compared with extracts-free control. The MIC values are reported as the mean of five experiments in triplicate.

Table 2. Bacterial strains used in the present study

Strains	Drug-resistance property	Reference
M. smegmatis		
MC^2 155	Wild type (sensitive to quinolones and fluoroquinolones)	Snapper et al. (1988)
MSR 101	Resistance to ciprofloxacin, lomofloxacin, norfloxacin	Sinha (2003)
MSR 102	Resistance to rifampicin, tetracycline, chloramphenicol	Sinha (2003)
MDR-40	Resistance to rifampicin, ampicillin, kanamycin	Singh (2004)
MDR-75	Resistance to isoniazid, streptomycin, cephalosporin	Singh (2004)
$CSMC^2$ 105	Resistance to ciprofloxacin	Srivastava (2002)
$CSLMC^2$ 205	Resistance to lomofloxacin	Srivastava (2002)
E. coli		
CA 8000	Wild type (sensitive to quinolones and fluoroquinolones)	Kumar (1976)
NK 5819	Resistance to nalidixic acid	Santha et al. (2000)

Results

Roots and inflorescence of two different genotypes (*gulabi* and KS-1) of *Vetiveria zizanioides* plants were collected from the research farm of the Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow, India. The hexane extract of root and inflorescence were tested for antibacterial activity against wild-type and six drug-resistant strains of *M. smegmatis* and one drug-resistant strain of *E. coli* using the disk diffusion and micro-broth dilution assays.

The hexane extracts of the intact root of both varieties were active against all the drug-resistant strains of *M. smegmatis* and *E. coli*. Interestingly, the hexane extracts of the spent root of the genotypes *gulabi* and KS-1 were found to be active against all the drug-resistant strains of *M. smegmatis* and *E. coli*. However, hexane extracts of intact and spent root of both genotypes of *Vetiveria zizanioides* did not show activity against the wild-type strain (CA 8000) of *E. coli*. The inflorescence extracts of both genotypes did not show any activity against any of the bacterial strains used (Table 3).

The observed antibacterial activity against the drug-resistant strains of *M. smegmatis* and *E. coli* was quantified using the broth dilution assay in terms of minimum inhibitory concentration. The MIC of hexane extracts of the roots of *Vetiveria zizanioides* cv *gulabi* and KS-1 against wild-type and six drug-resistant strains of *M. smegmatis* ranged from 62.5 to 250 μg/ml, and that of drug-resistant strains of *E. coli* ranged from 0.5 mg/ml ml to 60 mg/ml (Table 4).

Discussion

The current study was undertaken with the objective of searching for phytomolecules active against drug-resistant bacterial strains with special emphasis on the quinolone and fluoroquinolone class of antibiotics. The observations indicate that the hexane extract of the roots

Table 3. Antibacterial activity of the extracts of root and inflorescence of *Vetiveria zizanioides* var *gulabi* and KS-1 (zone of inhibition in millimeters).[a]

Vetiveria zizanioides	*Mycobacterium smegmatis*							*Escherichia coli*	
	MC^2 155	MSR101	MSR102	MDR-40	MDR-75	$CSMC^2$ 105	$CSLMC^2$ 205	CA 8000	NK 5819
Var. *gulabi*									
Root (intact)	5	6	3	2	5	1	4	0	5
Root (spent)	5	4	8	4	5	4	0	0	3
Inflorescence	0	0	0	0	0	0	0	0	0
Var. KS-1									
Root (intact)	4	5	4	2	5	3	2	0	4
Root (spent)	6	3	4	1	3	3	2	0	3
Inflorescence	0	0	0	0	0	0	0	0	0
Ciprofloxacin	34	32	31	37	34	14	28	34	32
Lomofloxacin	39	30	33	37	31	11	0	29	31
Nalidixic acid	0	18	0	0	16	0	15	18	19
DMSO	0	0	0	0	0	0	0	0	0

[a]Values are mean of five experiments in triplicate.

Table 4. Minimum inhibitory concentration (MIC μg/ml) of hexane extracts of *Vetiveria zizanioides* varieties *gulabi* and KS-1 on wild-type and drug-resistant strains of *M. smegmatis* and *E. coli*.[a]

Vetiveria zizanioides	*Mycobacterium smegmatis*							*Escherichia coli*	
	MC^2 155	MSR101	MSR102	MDR-40	MDR-75	$CSMC^2$ 105	$CSLMC^2$ 205	CA 8000	NK 5819
Var. *gulabi*									
Root (intact)	62.5	62.5	125	62.5	125	125	15.625	>1000	1000
Root (spent)	62.5	31.25	62.5	62.5	125	62.5	31.25	>1000	1000
Var. KS-1									
Root (intact)	125	62.5	62.5	31.25	125	125	31.25	>1000	>500
Root (spent)	125	62.5	31.25	62.5	250	125	31.25	>1000	1000
Ciprofloxacin	0.78	3.125	3.125	>25	3.125	25	25	0.78	0.78
Lomofloxacin	0.78	<01	10	2.5	0.78	>50	>50	<01	0.78
Nalidixic acid	50	6.25	50	50	3.125	50	25	6.25	1.56

[a]Values are mean of five experiments in triplicate.

of two varieties of the aromatic plant *Vetiveria zizanioides*, namely, KS-1 and *gulabi*, was active against all the bacterial strains used in this study.

The current global scenario is that disease-causing bacterial strains are acquiring resistance to most of the antibiotics used for treating bacterial infections. The quinolone and fluoroquinolone class of antibiotics is the last resort to treat this type of infection. The chances of acquiring resistance against these antibiotics are higher. Therefore, it is imperative to search for structurally different antibacterial agents that can kill the strains with modified DNA gyr activity, which is the target of quinolone and fluoroquinolone drugs.

Our observations showed the potential of extract from spent roots of *Vetiveria zizanioides* against drug-resistant bacteria from both Gram-positive and Gram-negative groups. The MIC of the hexane extracts of the different parts of the vetiver plant clearly demonstrated the antibacterial activity of root extracts against the biological screen used. While these findings support earlier reports on the antibacterial (Gangrade et al., 1990; Hammer et al., 1999) and antifungal (Gangrade et al., 1991) activity present in the essential oil of vetiver plants, because intact roots were active, we report activity even in oil-free roots after distillation. Formulations containing oil and/or extracts of *Vetiveria zizanioides* have been reported to treat inflammatory bowel disease (Jagtap & Shirke, 2002), urinary tract infection (Pandey et al., 2001), and have been reported in making insect repellents (Jain et al., 1982).

The useful observation in this study, however, is that the hexane extract of spent roots after distilling the oil out was more active against the drug-resistant strains like MSR101 (resistant to most of the quinolone and fluoroquinolone drugs), $CSMC^2$ 105 (resistant to ciprofloxacin), and MSR102 (resistant to rifampicin, tetracycline) as compared with the extracts of intact roots. This finding has implication of isolating the active molecule useful in treating the drug-resistant infections from the waste of vetiver, a commercially important plant. A U.S. Patent for such activity in vetiver root extract fraction has been granted to our laboratory. The genotypic difference observed for the level of activity being more in one variety (*gulabi*) over another (KS-1) indicates the possibility of screening germplasm of *Vetiveria zizanioides* to identify the best source of antibacterial principles.

Acknowledgments

U.S. Patent no. 6,676,974, Bioactive hexane fraction from *Vetiveria zizanioides*.

We thank the Department of Biotechnology, Government of India, and Council of Scientific and Industrial Research, New Delhi, for financial assistance.

References

Bauer AW, Kirby WMM, Sherries JC, Turck M (1966): Antibiotic sensitivity testing by a standardised single disk method. *Am J Clin Pathol 45*: 493–496.

Blanchard JS (1996): Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Annu Rev Biochem 65*: 215–239.

Brown EM, Reeves DS (1997): Quinolones. In: O'Grady F, Lambert HP, Finch RG, Greenwood D, eds., *Antibiotics and Chemotherapy*, 7th ed. Edinburgh, Churchill Livingstone, pp. 419–452.

Gangrade SK, Shrivastava RD, Sharma OP, Jain NK, Trivedi KC (1991): *In vitro* antifungal effect of the essential oils. *Indian Perfumer 35*: 46–48.

Gangrade SK, Shrivastava RD, Sharma OP, Moghe MN, Trivedi KC (1990): Evaluation of some essential oils for antibacterial properties. *Indian Perfumer 34*: 204–208.

Greenwood D (1997): Historical introduction. In: O'Grady F, Lambert HP, Finch RG, Greenwood D, eds.,

Antibiotics and Chemotherapy, 7th ed. Edinburgh, Churchill Livingstone, pp. 2–9.

Hammer KA, Carson CF, Riley TV (1999): Antimicrobial activity of essential oils and other plant extracts. *J App Microbiol 86*: 985–990.

Jagtap AG, Shirke SS (2002): Effect of polyherbal formulations on experimental models for inflammatory bowel disease. *Indian J Pharmacol 34*: 296–299.

Jain SK (1991): *Dictionary of Indian Folk Medicine and Ethnobotany*. New Delhi, Deep Publication, pp. 193–223.

Jain SC, Nowicki S, Eisner T, Meinwald J (1982): Insect repellents from vetiver oil: Zizanal and epizizanal. *Tetrahedron Lett 23*: 4639–4642.

Jorgensson JH, Turnigde DJ, Washington JA (1999): Antibacterial susceptibility tests: Dilution and disc diffusion methods. In: Murray PR, Barone EJ, P Faller MA, Tenover FC, Yolken RH, eds., *Manual of Clinical Microbiology*, 7th ed. Washington, DC, The American Society of Microbiology Press, pp. 1526–1543.

Kumar S (1976): Properties of adenyl cyclase and cyclic adenosine 3′–5′ monophosphate receptor protein deficient mutants of *Escherichia coli. J Bacteriol 125*: 545–555.

Nathan C (2004): Antibiotics at the cross roads. *Nature 431*: 899–902.

Pan X-S, Ambler J, Mehtar S, Fisher ML (1996): Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother 40*: 2321–2326.

Pandey KK, Vandana, Dwivedi M (2001): Urinary tract infection and its management by Renalka. *Antiseptic 98*: 295–296.

Projan SJ (2003): Why is Big Pharma getting out of antibacterial drug discovery? *Curr Opin Microbiol 6*: 427–430.

Rao RR, Suseela MR (2000): Vetiveria zizaniodes (Linn.) Nash. A multipurpose ecofriendly grass of India. Proceedings of Second International Conference on Vetiver. Office of the Royal Development Projects Board, Bangkok, pp. 444–448.

Santha Kumar TR, Khanuja SPS, Jain DC, Bhattacharya AK, Sharma RP, Kumar S (2000): A simple microbiological assay for the stereospecific differentiation of α and β isomers of arteether. *Phytother Res 14*: 644–646.

Sinha P (2003): Isolating plant derived antimycobacterial compounds through construction of novel target based bioscreens. Ph.D. Thesis, Barkatullah University, Bhopal, India.

Singh M (2004): Isolation and characterisation of multi drug resistant mutants in *Mycobacterium smegmatis*. M.Sc. Thesis, Barkatullah University, Bhopal, India.

Singh KK, Maheswari JK (1983): Traditional phytotherapy amongst the tribals of Varanasi district U.P. *J Econ Tax Bot 4*: 829–838.

Snapper SB, Lugosi L, Jekkel A, Melton RE, Kieser T, Bloom BR, Jacobs WR (1988): Lysogeny and transformation in *Mycobacteria*: Stable expression of foreign genes. *Proc Natl Acad Sci USA 85*: 6987–6991.

Srivastava S (2002): Bioprospecting potent plant compounds targeted to inhibit cell wall synthesis and DNA replication in *Mycobacterium smegmatis*. Ph.D. Thesis, Devi Ahilya Vishwavidyalaya, Indore, India.

Watt B (1997): *In vitro* sensitivities and treatment of less common mycobacteria. *J Antimicrob Chemother 39*: 567–574.

Wenzel RP (2004): The antibiotic pipeline-challenges, costs and values. *N Engl J Med 351*: 523–526.