

Adoption of ISSR fingerprinting for determination of sex using three primers, however, resulted in one female-specific band in the case of primer (GACA)₄, which was present in all the female and hermaphrodite plants but was absent in male plants of papaya (Figure 1). The two other ISSR primers used for the present study ((CAG)₅, (CAA)₅), however, did not result in any alteration of banding profile in male, female or hermaphrodite plants. Male-specific ISSR marker, however, has been reported¹² in papaya using primer (GATA)_n. Recent studies indicate that sex in papaya is governed by a single gene with three alleles¹³. A high-density linkage map of papaya revealed severe suppression of recombination around the sex-determination locus with a total of 225 markers co-segregating with sex types¹⁴. In terms of agriculture, the discovery of markers linked to sex chromosomes will help farmers to selectively grow hermaphrodite papayas.

Sexual dimorphism of *C. circinalis* was readily distinguishable in RAPD profiles generated from a number of random primers under study, among which the profiles of primers OPB 01 and OPB 05 were noteworthy, since they represent one male-specific (686 bp) and another female-specific (2048 bp) band respectively (Figure 2). Sequencing of these two cloned DNA fragments, followed by BLASTX searching, revealed maximum homology with reverse transcriptase of *Ginkgo biloba* (score 69.3 bits; NCBI accession no. AAY87195) followed by putative retroelement pol poly protein of *Arabidopsis thaliana* (score 59.3 bits; NCBI accession no. AAC61290) and putative poly protein of *Oryza sativa* (japonica cultivar-group; score 58.5 bits; NCBI accession no. AAU90089) in case of male-specific DNA fragment (NCBI accession DQ386640, dated 22.02.2006), while the female-specific DNA fragment did not result in any significant match. Homology of male-specific sequence with retroelement calls for further investigation towards isolation of full-length sequence of this DNA fragment to be used as a marker for male *C. circinalis* in future. Diversity, evolution and genome organization of retroelements have been studied in a wide range of gymnosperms, especially the conifers^{15,16}, but assigning of direct relationship to sex is yet to be established.

7. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
8. Bornet, B. and Branchard, M., Nonanchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. *Plant Mol. Biol. Rep.*, 2001, **19**, 209–215.
9. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J., Basic local alignment search tool. *J. Mol. Biol.*, 1990, **215**, 403–410.
10. Schlotterer, C., The evolution of molecular markers – just a matter of fashion? *Nature Rev. Genet.*, 2004, **5**, 63–69.
11. Banerjee, N. S., Manoj, P. and Das, M. R., Male sex associated RAPD markers in *Piper longum* L. *Curr. Sci.*, 1999, **77**, 693–695.
12. Parasnis, A. S., Ramakrishna, W., Chowdari, K. V., Gupta, V. S. and Ranjekar, P. K., Microsatellite (GATA)_n reveals sex specific differences in papaya. *Theor. Appl. Genet.*, 1999, **99**, 1047–1052.
13. Liu, Z. Y. *et al.*, A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature*, 2004, **427**, 348–352.
14. Ma, H. *et al.*, High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. *Genetics*, 2004, **166**, 419–436.
15. Friesen, N., Brandes, A. and Heslop-Harrison, J. S., Diversity, origin and distribution of retrotransposons (*gypsy* and *copia*) in conifers. *Mol. Biol. Evol.*, 2001, **18**, 1176–1188.
16. Murray, B. G., Friesen, N. and Heslop-Harrison, J. S., Molecular cytogenetic analysis of *Podocarpus* and comparison with other gymnosperm species. *Ann. Bot.*, 2002, **89**, 483–489.

ACKNOWLEDGEMENTS. We thank the Director, Bose Institute, Kolkata for help and encouragement. S.K.R. thanks DBT, New Delhi for financial support. The nucleotide sequence data reported here are available in the NCBI nucleotide sequence databases under the accession number DQ386640.

Received 3 March 2006; revised accepted 4 October 2006

Free-radical scavenging activities of Himalayan rhododendrons

Dhan Prakash^{1,*}, Garima Upadhyay¹,
B. N. Singh¹, Ruchi Dhakarey¹, Sandeep Kumar²
and K. K. Singh²

¹Nutraceutical Chemistry Laboratory, National Botanical Research Institute, Lucknow 226 001, India

²G. B. Pant Institute of Himalayan Environment and Development, Sikkim Unit, Pngethang-Gangtok, P.O., Penlong 737 102, India

Reactive oxygen species can damage cellular biomolecules leading to degenerative diseases. Phenols, a major group of phytochemicals with antioxidant properties, can help inactivate them. To find the antioxidant potential of the genus *Rhododendron*, its 21 species were studied for their total phenolic content (TPC), flavonoids and antioxidant activity (AOA). TPC varied from 37.3 to 208.9 mg/g, flavonoids from 11.5 to 137.1 mg/g and AOA from 30.4 to 97.4%. *R. baileyi*, *R. camellie-*

1. Bell, G., *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*, University of California Press, Maynard, 1982.
2. Tanurdzic, M. and Banks, J. A., Sex-determining mechanisms in land plants. *Plant Cell*, 2004, **16**, S61–S71.
3. Ainsworth, C., Crossley, S., Buchanan-Wollaston, V., Thangavelu, M. and Parker, J., Male and female flowers of the dioecious plant sorrel show different patterns of MADS box gene expression. *Plant Cell*, 1995, **7**, 1583–1598.
4. Magdalita, P. M. and Mercado, C. P., Determining the sex of papaya for improved production. *Bull. Food Fert. Tech. Centre, Philippines*, 2003, 1–6 (<http://www.agnet.org/library/article/eb534.html>).
5. Deputy, J. *et al.*, Molecular markers for sex determination in papaya (*Carica papaya* L.). *Theor. Appl. Genet.*, 2002, **106**, 107–111.
6. Rogers, S. O. and Bendich, A. J., *Plant Molecular Biology Manual* (eds Gelvin, S. P. and Schilperoort, R. A.), Kluwer, Dordrecht, The Netherlands, 1988, p. A6: 1.

*For correspondence. (e-mail: dprakash_in@yahoo.com)

florum, *R. campanulatum*, *R. ciliatum*, *R. cinnabarinum*, *R. griffithianum*, *R. lepidotum*, *R. niveum*, *R. sallignum* and *R. virgatum* were found to have high TPC (91.4–208.9 mg/g), AOA (71.5–97.4%) and free radical scavenging activity, as evident from their low IC₅₀ (inhibitory concentration, 0.07–0.19 mg/ml), low EC₅₀ (efficiency concentration, 3.28–8.26 mg/mg), and high ARP (antiradical power, 12.10–30.48), compared to reference standard. *R. griffithianum*, *R. lepidotum* and *R. virgatum* showed better ferrous-ion chelating capacity and inhibition of lipid peroxidation than that of standards, BHT and quercetin. They also showed better reducing power and inhibition of both site-specific and non site-specific hydroxyl radicals-induced deoxyribose degradation than those of other species. *R. camellieflorum*, *R. campanulatum*, *R. griffithianum*, *R. lepidotum* and *R. virgatum* were potential scavengers of superoxide anions and also showed significant protection of DNA damage induced by free radicals. Promising species were also subjected to HPLC and MS/MS, which showed the presence of phenolic acids (gallic, caffeic, chlorogenic, ellagic and protocatechuic acids) and flavonoids (quercetin, kaempferol and rutin).

Keywords: Antioxidant activity, free radicals, rhododendrons, scavenging, total phenolic content.

THE genus *Rhododendron* (Greek Rhodon = rose and dendron = tree), family Ericaceae, ranges in size from a few centimetres (a tiny mat-like growth in the alpine region, *R. setosum*), to giants up to 25 m (*R. arboreum*). Slow growth rate is its characteristic feature. The genus has about 50 species in India¹ mainly distributed in the Himalayan region, while *R. nilagiricum* is the only species found in South India. About 98% of the Indian species is found in the Himalayas, out of which 72% occurs in Sikkim. In Sikkim Himalayas, *Rhododendron* species show a barrel-shaped altitudinal distribution¹. Apart from their worldwide aesthetic and ethnic uses, several species have commercial and medicinal values². Chemical constituents of *R. dauricum*, a traditional Chinese medicinal herb, were reported along with their pharmaceutical importance³. Kashiwada *et al.*⁴ reported daurichromanin acid, rhododaurichromanin acid A and B from its leaves and twigs; the first two showed anti-HIV activity. Chosson *et al.*⁵ found six flavonoids and their glycosides from the leaves and flowers of *R. ferrugineum*. Rhodojaponin III, grayanotoxin III and kalmanol, three grayanoid diterpenes were identified as the most active insecticidal constituents in dried flowers of *R. molle*⁶. Anthocyanins and antioxidants from flowers and leaves of *R. simsii* and other species have been reported^{7,8}. Leaf composition of 206 species, subspecies and varieties showed simple phenols in 55 species, with a relatively uniform flavonoid pattern^{9,10}.

Reactive oxygen species are known to damage cellular biomolecules, resulting in several diseases. Antioxidants play a key role to scavenge free radicals and are associated

with reduced risk of cancer and cardiovascular diseases¹¹. Due to a wide range of applications and to find their antioxidant potential, 21 species of *Rhododendron* were studied for their phenols, phenolic composition, free radical scavenging activities and protection of DNA damage.

Leaves of 21 species of *Rhododendron* collected from different geographical regions of Sikkim Himalayas were chopped, dried, powdered (40-mesh) and stored in polythene bags in a refrigerator till analysis. The plant material (1.0 g) was extracted with 50% MeOH : H₂O (2 × 20 ml), overnight at room temperature. Total phenolic content (TPC) in the extracts was measured by the method of Ragazzi and Veronese¹², and expressed as mg gallic acid equivalent (GAE)/g sample on dry weight basis. Total flavonoids were estimated as described by Oyaizu¹³ and expressed as mg quercetin equivalent (QE)/g sample. The antioxidant activity (AOA) of extracts was studied by auto oxidation of β -carotene and linoleic acid-coupled reaction according to Emmons and Peterson¹⁴ and was expressed as per cent inhibition relative to control. AOA was also determined by ammonium thiocyanate assay¹⁵. Free radical scavenging activity (FRSA) was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical¹⁶. The inhibitory concentration (IC₅₀), efficiency concentration (EC₅₀) and antiradical power (ARP) were estimated and calculated as described by Kroyer¹⁷. Reducing capacity of extracts was determined¹⁸ (ASE/ml = absorbance of 1 mM ascorbic acid/absorbance of 1 mg/ml sample) by ferric reducing-antioxidant power assay using quercetin as reference standard and expressed as ascorbic acid equivalent (1 mM = 1 ASE). Hydroxyl radical scavenging activity was measured¹⁹ and the degree of deoxyribose oxidation was analysed as thiobarbituric acid reactive species. Chelating capacity of ferrous ions was estimated²⁰ and superoxide radical scavenging activity was assayed²¹ by reduction of nitroblue tetrazolium chloride (NBT). DNA nicking assay were performed using supercoiled pBR322 DNA by the method of Lee *et al.*¹⁵ and analysed on 1% agarose gel. Qualitative and quantitative analysis was performed using Shimadzu LC-10A (Japan) HPLC system equipped with dual pump, UV detector at 254 nm, Phenomenex Luna RP, C18 column (4.6 × 250 mm) with linear gradient of a solvent system comprising acetonitrile and water containing 1% acetic acid. Data were integrated by Shimadzu class VP series software and results were obtained by comparison with standards. Qualitative analysis was further confirmed by API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) on a turbo ions spray source in negative mode. MS/MS product ions were produced by collision-associated dissociation of the selected precursor ions in a collision cell. In all the experiments, the quadrupole (Q₁) was operated at unit resolution. Product ion scan of selected molecules was carried out in order to confirm the structure of the compounds. Results are the mean values of three replicates of

RESEARCH COMMUNICATIONS

Table 1. Moisture (%), total phenolic content (TPC, mg gallic acid equivalent/g sample), flavonoids (mg quercetin equivalent/g sample) and antioxidant activity (AOA, %) measured by auto-oxidation of β -carotene and linoleic acid-coupled reaction of some *Rhododendron* species on dry wt basis

Species	English name*	Moisture	TPC	Flavonoids	AOA
<i>Rhododendron arboreum</i> arb.	Scarlet arborescent Rd	66.3	57.3	43.8	54.2
<i>R. arboreum</i> cinn.	Scarlet arborescent Rd	59.3	78.5	62.6	81.6
<i>R. baileyi</i>	Bailey's Rd	69.3	97.9	32.0	92.0
<i>R. barbatum</i>	Bristly Rd	75.2	44.1	11.5	66.5
<i>R. camellieflorum</i>	Camellia-flowered Rd	61.6	132.2	59.1	93.6
<i>R. campanulatum</i>	Bell-flowered Rd	73.5	123.9	24.5	94.4
<i>R. ciliatum</i>	Ciliated Rd	73.9	91.4	88.4	71.5
<i>R. cinnabarinum</i>	Cinnabar Rd	61.2	93.9	26.3	83.2
<i>R. dalhousiae</i>	Lady Dalhousie's Rd	72.9	55.4	19.6	57.2
<i>R. decipiens</i>	Lady Dalhousie's Rd	76.6	39.6	23.2	55.0
<i>R. falconerii</i>	Dr Falconer's Rd	64.9	39.2	21.1	30.4
<i>R. grande</i>	Large silvery Rd	65.2	37.3	41.4	56.4
<i>R. griffithianum</i>	Lord Auckland's Rd	68.4	165.4	77.3	93.4
<i>R. lepidotum</i>	Scaly Rd	68.8	148.5	94.5	87.2
<i>R. maddenii</i>	Major Madden's Rd	67.5	87.3	54.4	62.8
<i>R. niveum</i>	Snow-leaved Rd	58.2	106.6	57.1	85.2
<i>R. pendulum</i>	Pendulous Rd	72.3	89.3	65.2	76.8
<i>R. salignum</i>	Saligna Rd	66.1	97.5	34.8	88.4
<i>R. thomsonii</i>	Dr Thomson's Rd	74.6	90.6	22.1	90.0
<i>R. vaccinioides</i>	Vaccinium-like Rd	53.5	67.8	14.7	72.0
<i>R. virgatum</i>	Twiggy Rd	57.1	208.9	137.1	97.4
LSD at $P < 0.01$		0.66	2.61	0.72	1.44

*Source: Pradhan and Lachungpa²⁸.

Rd, Rhododendron; AOA % = $100 \times (\text{DRc} - \text{DRs}) / \text{DRc}$, where DRc is the rate of degradation of control and DRs the rate of degradation of sample.

the same sample and statistical analysis was performed using analysis of variance.

Phenols and flavonoids are known to be responsible for FRSA. To find the antioxidant potential, 21 species of *Rhododendron* were studied (Table 1) for their total TPC, flavonoids and AOA. The contents of moisture varied from 53.5 (*R. vaccinioides*) to 76.6% (*R. decipiens*), TPC from 37.3 (*R. grande*) to 208.9 mg GAE/g (*R. virgatum*), flavonoids from 11.5 (*R. barbatum*) to 137.1 mg QE/g (*R. virgatum*) and AOA measured by auto-oxidation of β -carotene and linoleic acid-coupled reaction ranged from 30.4 (*R. falconerii*) to 97.4% (*R. virgatum*). Total flavonoids in most of the species were low; however, *R. ciliatum*, *R. lepidotum* and *R. virgatum* showed promising quantities (88.4–137.1 mg/g). A seasonal variation of 2.6 to 4.0% of flavonoids in *R. dauricum* had been reported³. *R. baileyi*, *R. camellieflorum*, *R. campanulatum*, *R. ciliatum*, *R. cinnabarinum*, *R. griffithianum*, *R. lepidotum*, *R. niveum*, *R. salignum* and *R. virgatum* were found to have reasonably good amounts of total phenols (91.4–208.9 mg GAE/g) that might be responsible for the high antioxidant activity (71.5–97.4%). This was further substantiated by FRSA measured using DPPH free radical assay (Table 2), where the latter is reduced to the corresponding hydrazine by the test sample. The reasonably good efficiency as free radical scavengers was evident by their low IC_{50} (0.07 to 0.19 mg/ml), low EC_{50} (3.28 to 8.26 mg/mg DPPH) and high ARP (12.10 to 30.48) than the reference standard.

The high reducing power (0.46–1.43 ASE/ml) indicated their potential as electron donors to scavenge free radicals efficiently. Promising species were further subjected to concentration-dependent FRSA using five different methods and expressed in terms of IC_{50} values (Table 3). The IC_{50} values for inhibition of lipid peroxidation measured by ammonium thiocyanate assay ranged from 0.67 to 2.77 mg/ml; *R. virgatum* (0.67 mg/ml), *R. griffithianum* (0.84 mg/ml) and *R. lepidotum* (1.15 mg/ml) showed better inhibition of peroxide formation compared to reference standards, BHT (1.27 mg/ml) and quercetin (1.85 mg/ml). The 40.35 and 56% inhibition of lipid peroxidation had been reported in water and ethanol extracts (2 mg/ml) of ginger respectively²². These species also showed better non site-specific and site-specific inhibition of hydroxyl radical-induced deoxyribose degradation as evident by their low IC_{50} values than the rest of the species (Table 3). The non site-specific scavengers would compete with deoxyribose for availability of hydroxyl radicals, resulting in the reduction of rate of reaction. On the other hand, site-specific scavengers would offer protection by chelating with ferrous ions. Hydroxyl radical scavenging activity of 40.1 and 75% respectively, in extracts of ginger²² at a concentration of 3.0 mg/ml, and potato peel²³ at 5.0 mg/ml were reported. *R. griffithianum* (0.40 mg/ml), *R. lepidotum* (0.62 mg/ml) and *R. virgatum* (0.31 mg/ml) were also found as potential superoxide anions scavengers evaluated by inhibitory capabilities of NBT reduction that

Table 2. Free radical scavenging activity of some *Rhododendron* species measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in terms of IC₅₀ (inhibitory concentration; mg/ml of extract), EC₅₀ (efficiency concentration; mg/mg DPPH); ARP (antiradical power) and reducing power (ASE/ml)

Species	IC ₅₀	EC ₅₀	ARP	ASE/ml
<i>R. arboreum arb.</i>	0.47	20.59	4.85	1.23
<i>R. arboreum cinn.</i>	0.34	14.78	6.76	1.25
<i>R. baileyi</i>	0.14	6.11	16.35	1.09
<i>R. barbatum</i>	0.64	28.16	3.54	1.21
<i>R. camellieflorum</i>	0.12	5.32	18.77	1.26
<i>R. campanulatum</i>	0.13	5.65	17.69	0.71
<i>R. ciliatum</i>	0.19	8.26	12.10	1.43
<i>R. cinnabarinum</i>	0.16	6.21	16.10	1.57
<i>R. dalhousiae</i>	0.51	22.58	4.42	1.11
<i>R. decipiens</i>	0.72	31.40	3.12	1.71
<i>R. falconerii</i>	0.82	36.21	2.78	1.63
<i>R. grande</i>	0.86	37.39	2.67	1.61
<i>R. griffithianum</i>	0.10	4.35	22.93	0.64
<i>R. lepidotum</i>	0.12	5.28	18.92	1.00
<i>R. maddenii</i>	0.30	13.04	7.66	1.31
<i>R. niveum</i>	0.13	5.70	17.52	0.72
<i>R. pendulum</i>	0.22	9.88	10.12	1.72
<i>R. sallignum</i>	0.14	6.18	16.15	1.58
<i>R. thomsonii</i>	0.22	9.56	10.46	2.03
<i>R. vaccinioides</i>	0.45	19.56	5.19	1.16
<i>R. virgatum</i>	0.07	3.28	30.48	0.46
Quercetin, standard	0.20	8.6	11.6	0.5
LSD at $P < 0.01$	0.049	2.18	6.24	0.158

EC₅₀ = IC₅₀/concentration of DPPH in mg/ml; ARP = 100/EC₅₀; ASE = ferric reducing-antioxidant power expressed as ascorbic acid equivalent (1 mM = 1 ASE), which is inversely proportional to reducing power.

Table 3. FRSA of some promising *Rhododendron* species measured by different methods and expressed as inhibitory concentration (IC₅₀, mg/ml)

Species	A	B	C	D	E
<i>R. baileyi</i>	2.77	3.50	2.54	2.04	2.56
<i>R. camellieflorum</i>	1.42	1.91	1.36	0.73	0.70
<i>R. ciliatum</i>	2.47	2.55	2.08	1.75	2.04
<i>R. cinnabarinum</i>	1.78	2.01	1.42	0.97	0.91
<i>R. campanulatum</i>	1.51	2.21	1.55	0.78	0.83
<i>R. griffithianum</i>	0.84	1.62	1.12	0.39	0.62
<i>R. lepidotum</i>	1.15	1.82	1.24	0.62	0.60
<i>R. niveum</i>	1.64	1.94	1.37	0.67	0.85
<i>R. sallignum</i>	2.11	2.43	1.85	0.87	2.19
<i>R. virgatum</i>	0.67	1.60	1.19	0.31	0.43
BHT	1.27	1.53	0.45	0.51	1.81
Quercetin	1.85	0.68	1.06	1.20	0.66
LSD at $P < 0.01$	2.86	2.01	2.82	1.41	2.06

A, Hydroxyl radical scavenging activity assayed by ammonium thiocyanate method; B, Non-site specific inhibition of hydroxyl radical-mediated deoxy ribose degradation; C, Site-specific inhibition of hydroxyl radical-mediated deoxyribose degradation; D, Inhibition of NBT reduction caused by superoxide anions and E, Ferrous ion chelating capacity.

showed IC₅₀ values lower or in close proximity with BHT (0.51 mg/ml). The scavenging effect of superoxide anions in ethanol (80.9%) and water (77.7%) extracts of ginger²² and potato peel²³ (60 to 84%; at concentrations of 0.5 to 5.0 mg/ml) have been mentioned. The ferrous ion-chelating capacity of *R. griffithianum* (0.62 mg/ml), *R. lepidotum* (0.60 mg/ml) and *R. virgatum* (0.43 mg/ml) was also

better compared to BHT (1.81 mg/ml) and quercetin (0.66 mg/ml). Transition metal ions are known to catalyse the formation of free radicals. On the other hand, phenolic compounds can inhibit their formation by chelating with metal ions. Extracts of Holy basil²⁴ showed ferrous che-

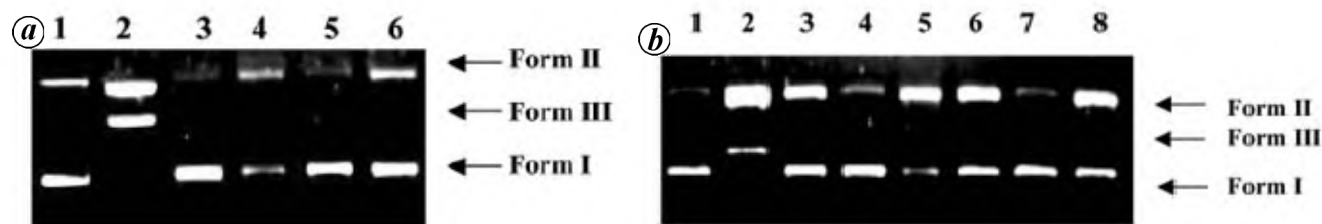


Figure 1. *a*, Concentration-dependent inhibitory effects of *Rhododendron virgatum* leaf extracts ($\mu\text{g/ml}$) on native pBR322 DNA nicking caused by hydroxyl radicals. Lane 1, Native DNA; lane 2, DNA + Fenton reagent; lane 3, DNA + Fenton reagent + 2U Catalase; lane 4, DNA + Fenton reagent + 5 $\mu\text{g/ml}$ extract; lane 5, DNA + Fenton reagent + 10 $\mu\text{g/ml}$ extract and lane 6, DNA + Fenton reagent + 20 $\mu\text{g/ml}$ extract. *b*, Inhibitory effects of plant extracts (20 $\mu\text{g/ml}$) on native pBR322 DNA nicking caused by hydroxyl radicals. Lane 1, Native DNA; lane 2, DNA + Fenton reagent; lane 3, DNA + Fenton reagent + 2U Catalase; lane 4, DNA + Fenton reagent + *R. griffithianum*; lane 5, DNA + Fenton reagent + *R. cilliatum*; lane 6, DNA + Fenton reagent + *R. campanulatum*; lane 7, DNA + Fenton reagent + *R. lepidotum* and lane 8, DNA + Fenton reagent + *R. camelliflorum*.

Table 4. Specific phenolic composition ($\mu\text{g/g}$) of some *Rhododendron* species estimated using HPLC

Species	CA	CHL	EA	GA	PCA	KMP	QC	RT
<i>R. baileyi</i>	–	–	–	467.8	8.7	–	–	–
<i>R. camelliflorum</i>	–	102.5	62.5	246.5	–	–	333.7	–
<i>R. cilliatum</i>	88.3	42.9	7.1	162.6	188.2	–	152.6	62.2
<i>R. cinnabarinum</i>	–	324.6	27.1	158.2	–	–	174.2	–
<i>R. campanulatum</i>	–	190.2	–	279.8	42.1	–	193.4	–
<i>R. griffithianum</i>	–	282.4	36.3	328.3	53.5	–	392.5	–
<i>R. lepidotum</i>	50.9	349.7	81.2	360.7	119.5	–	674.5	–
<i>R. maddenii</i>	–	–	–	291.5	–	100.1	112.4	–
<i>R. niveum</i>	15.9	78.5	–	475.3	–	–	245.8	–
<i>R. salignum</i>	4.6	–	–	203.1	51.8	–	–	–
<i>R. thomsonii</i>	–	–	–	416.4	64.5	–	671.8	–
<i>R. virgatum</i>	76.9	335.7	69.5	679.6	168.7	–	663.9	–

CA, Caffeic acid; CHL, Chlorogenic acid; EA, Ellagic acid; GA, Gallic acid; PCA, Protocatechuic acid; KMP, Kaempferol; QC, Quercetin and RT, Rutin.

lating capacity of 33.31 and 32.05% at a concentration of 0.75 and 1.0 mg/ml, while potato peel²³ showed ferrous chelating capacity of 50% at 5.0 mg/ml. The concentration-dependent (5.0 to 20 $\mu\text{g/ml}$) scavenging effects of *R. virgatum* extract on Fe^{3+} -induced free hydroxyl radicals showed significant protection of DNA damage (Figure 1 *a*) and mitigated oxidative stress. Extracts of *R. campanulatum*, *R. griffithianum*, *R. lepidotum* and *R. virgatum* at 20.0 $\mu\text{g/ml}$ showed reduction in the formation of single-stranded nicked DNA (form II, circular), double-stranded nicked DNA (form III, linear) and increased DNA (form I, super-coiled) (Figure 1 *a* and *b*).

Promising species were subjected to HPLC to estimate their specific phenolic composition (Table 4), which showed the presence of caffeic acid, chlorogenic acid, ellagic acid, gallic acid, protocatechuic acid, kaempferol, quercetin and rutin. The quantities of caffeic acid varied from 4.6 to 88.3 $\mu\text{g/g}$, chlorogenic acid 42.9 to 349.7 $\mu\text{g/g}$, ellagic acid 7.1 to 81.2 $\mu\text{g/g}$, gallic acid 158.2 to 679.6 $\mu\text{g/g}$, protocatechuic acid 8.7 to 188.2 $\mu\text{g/g}$ and quercetin 112.4 to 674.5 $\mu\text{g/g}$. Identification of specific polyphenols was further substantiated by MS/MS analysis (Table 5) which showed the deprotonated molecule $[\text{M}-\text{H}]^-$. Loss of CO_2 was observed for caffeic, gallic and protocatechuic acids,

giving $[\text{M}-\text{H}-44]^-$ as a characteristic ion. Chlorogenic acid showed the deprotonated molecule $[\text{M}-\text{H}]^-$ at m/z 353 and ion corresponding to the deprotonated quinic acid at m/z 191. Ellagic acid, a dimer of gallic acid gave m/z at 170, the product ion scan of which exhibited a characteristic ion fragment of m/z 125 showing loss of CO_2 . Flavonol O-glycosides such as rutin showed the deprotonated molecule of the glycoside and ion corresponding to the deprotonated aglycone $[\text{A}-\text{H}]^-$. The latter ion was formed by the loss of rhamnose and glucose from the glycosides. Finally, aglycones such as quercetin and kaempferol gave retro-Diels–Alder fragmentation where m/z 151 was common, but in case of kaempferol loss of neutral water molecule also afforded m/z at 133.

The identified phenolic compounds have well documented FRSA activities and metal ion-chelating capacity. Gallic acid, present in good quantities in the *Rhododendron* species had been reported²⁵ to have high FRSA compared to rutin, ferulic acid, tannic acid, caffeic acid, BHA, resveratrol, tocopherol, etc. It has also been found that plant extracts containing flavonoids and chlorogenic acid are highly effective in scavenging DPPH radical¹⁸, superoxide anion radical²⁶ and in metal chelating capacity²⁷. Similarly, the mechanism of action of quercetin

Table 5. Phenolic composition of some *Rhododendron* species identified by MS/MS

Phenols	Species	Ion full scan MS		MS/MS approach
		[M-H] ⁻	Fragments	Product ion scan
Protocatechuic acid	A, C, E, F, G, J, K, L	153	109	153
Caffeic acid	C, G, I, J, L	179	135	179
Chlorogenic acid	B, C, D, E, F, G, I, L	353	191	353
Ellagic acid	B, C, D, F, G, L	301	125, 170	170
Gallic acid	A, B, C, D, E, F, G, H, I, J, K, L	169	125	169
Kaempferol	H	285	133, 151	285
Quercetin	B, C, D, E, F, G, H, I, K, L	301	151	301
Rutin	C	609	301	609

A, *R. baileyi*; B, *R. camellieflorum*; C, *R. ciliatum*; D, *R. cinnabarium*; E, *R. companulatum*; F, *R. griffithianum*; G, *R. lepidotum*; H, *R. maddenii*; I, *R. niveum*; J, *R. salignum*; K, *R. thomsonii* and L, *R. virgatum*.

also includes free radical scavenging, chelation of metal ions and inhibition of lipid peroxidation. Chlorogenic acid, gallic acid and quercetin that were present in appreciable quantities in *Rhododendron* species, along with other phenols might be responsible for their efficient FRSA. Reactive oxygen species can cause damage to cellular biomolecules like DNA, RNA, enzymes, lipids, carbohydrates and consequently may adversely affect immune functions. Oxidation of bases in DNA, deoxyribose lesions and strand breaks may lead to mutagenic changes and a variety of diseases. Phenols, due to their strong antioxidants and a range of biological properties, are also known to diffuse the toxic free radicals^{11,15,18}.

From the foregoing it may be concluded that *Rhododendron* species with high levels of phenols, promising antioxidant and free radical scavenging activities may be utilized in the development of healthcare products and/or for the isolation of specific desired phytochemical(s).

1. Singh, K. K., Kumar, S., Rai, L. K. and Krishna, A. P., *Rhododendron* conservation in Sikkim Himalayas. *Curr. Sci.*, 2003, **85**, 602–606.
2. Leach, D. G., The ancient course revisited. *Himalayan Plant J.*, 1986, **4**, 69–72.
3. Cao, Y., Chu, Q. and Ye, J., Biomedically relevant plant components: Active principles and toxicants, chromatographic and electrophoretic methods for pharmaceutically active compounds in *Rhododendron dauricum*. *J. Chromatogr. B*, 2004, **812**, 231–240.
4. Kashiwada, Y. *et al.*, Isolation of rhododaurichroman acid B and the anti-HIV principles rhododaurichroman acid A and rhododaurichroman acid from *Rhododendron dauricum*. *Tetrahedron*, 2001, **57**, 1559–1563.
5. Chosson, E., Chaboud, A., Chulia, A. J. and Raynaud, J., Dihydroflavonol glycosides from *Rhododendron ferrugineum*. *Phytochemistry*, 1998, **49**, 1431–1433.
6. Klocke, J. A., Hu, M., Chiu, S. and Kubo, I., Grayanoid diterpene insect antifeedants and insecticides from *Rhododendron molle*. *Phytochemistry*, 1991, **30**, 1797–1800.
7. de Loose, R., Flavonoid glycosides in the petals of some *Rhododendron* species and hybrids. *Phytochemistry*, 1970, **9**, 875–879.
8. Takahashi, H., Hirata, S., Minami, H. and Fukuyama, Y., Triterpene and flavanone glycoside from *Rhododendron simsii*. *Phytochemistry*, 2001, **56**, 875–879.

9. Harborne, J. B. and Williams, C. A., Leaf survey of flavonoids and simple phenols in the genus *Rhododendron*. *Phytochemistry*, 1971, **10**, 2727–2744.
10. Harborne, J. B., Flavonoid patterns and phytochemistry: The genus *Rhododendron* section *Vireya*. *Phytochemistry*, 1986, **25**, 1641–1643.
11. Willcox, J. K., Ash, S. L. and Catignani, G. L., Antioxidants and prevention of chronic disease. *Crit. Rev. Food Sci. Nutr.*, 2004, **44**, 275–295.
12. Ragazzi, E. and Veronese, G., Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *J. Chromatogr.*, 1973, **77**, 369–375.
13. Oyaizu, M., Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. *Jpn. J. Nutr.*, 1986, **44**, 307–315.
14. Emmons, C. L. and Peterson, D. M., Antioxidant activity and phenolic contents of oats, groats and hulls. *Cereal Chem.*, 1999, **76**, 902–905.
15. Lee, J. C., Kim, H. R., Kim, J. and Jang, Y. S., Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. *J. Agric. Food Chem.*, 2002, **50**, 6490–6496.
16. Yen, G. C. and Duh, P. D., Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. *J. Agric. Food Chem.*, 1994, **42**, 629–632.
17. Kroyer, G. T., Red clover extract as antioxidant active and functional food ingredient. *Innov. Food Sci. Emerg. Technol.*, 2004, **5**, 101–105.
18. Apati, P., Szentmihályi, K., Kristo, Sz. T., Papp, I., Vinkler, P., Szoke, E. and Kery, A., Herbal remedies of Solidago, correlation of phytochemical characteristics and antioxidative properties. *J. Pharm. Biomed. Anal.*, 2003, **32**, 1045–1053.
19. Halliwell, B., Gutteridge, J. M. C. and Aruoma, O. I., The deoxyribose method: a simple test tube assay for the determination of rate constants for the reaction of hydroxy radicals. *Anal. Biochem.*, 1987, **165**, 215–219.
20. Decker, E. A. and Welch, B., Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.*, 1990, **38**, 674–678.
21. Nishikimi, M., Rao, N. A. and Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 1972, **46**, 849–864.
22. Lee, J. C. and Lim, K. T., Effect of cactus and ginger extracts as dietary antioxidant on reactive oxidant and plasma lipid level. *Food Sci. Biotechnol.*, 2000, **9**, 83–88.
23. Singh, N. and Raginia, P. S., Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.*, 2004, **85**, 611–616.
24. Juntachote, T. and Berghofer, E., Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal. *Food Chem.*, 2005, **92**, 193–205.

25. Sanchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F., Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res. Int.*, 1999, **32**, 407–412.
26. Kim, M. Y., Iwai, K. and Matsue, H., Phenolic composition of *Viburnum dilatatum* Thunb. fruits and their antiradical properties. *J. Food Comp. Anal.*, 2005, **18**, 789–802.
27. Lean, M., Norrozi, M., Kelly, L., Burns, J., Talwar, D., Satter, N. and Crozier, A., Dietary flavanoids protect diabetic human lymphocytes against oxidant damage to DNA. *Diabetes*, 1999, **48**, 176–181.
28. Pradhan, U. C. and Lachungpa, S. T., In *Sikkim Himalayan Rhododendrons*, Primulaceae Books, Kalimpong, 1990.

ACKNOWLEDGEMENTS. We thank Dr Rakesh Tuli, Director, National Botanical Research Institute, Lucknow for his interest, encouragement and providing research facilities and Dr Abhishek Niranjana for LC-MS/MS.

Received 15 December 2005; revised accepted 20 September 2006

Active faults and neotectonic activity in the Pinjaur Dun, northwestern Frontal Himalaya

G. Philip* and N. S. Virdi

Wadia Institute of Himalayan Geology, 33, Gen. Mahadeo Singh Road, Dehra Dun 248 001, India

Manifestation of ongoing active tectonics in the Himalayan foothill region is evident from a number of major $M > 7.5$ and great earthquakes ($M \approx 8$) which occurred within the last century or so, besides the recurrent seismic events of moderate magnitude. Active faults of the Himalaya are significant in the study of active tectonics because displacements along them reflect the continued tectonic movements. The present study deals with morpho-structural analysis using remotely sensed data along with selected field investigations in delineating new traces of active faults in the Pinjaur Dun of the northwestern Frontal Himalaya. Fault scarps with heights varying from 5 to 25 m observed along the faults are indicative of long term uplift/deformation in the current tectonic regime and cumulative slip along them. These active faults are signatures of Quaternary tectonics in the zone between the Main Boundary Thrust (MBT) and the Himalayan Frontal Thrust.

Keywords: Active faults, Frontal Himalaya, neotectonics, Pinjaur Dun, remote sensing.

ACTIVE faults are widely distributed in different sectors of the Himalaya and are important in that they provide signa-

tures of the recurrent tectonic activity during the Quaternary and in particular the Holocene periods. The activity often resulted in destructive earthquakes, dislocation of old landforms and creation of new ones. Landforms such as river terraces, alluvial fans, fault scarps and other morpho-tectonic features such as triangular facets, knick points, sag ponds, shutter ridges, pressure ridges and pull-apart basins, controlled drainage, and stream piracy are closely related with activity along these active faults^{1–10}. Geomorphic and morphotectonic analyses of landforms provide insights into rates, style and pattern of deformation due to active tectonics.

Geodetic, GPS and seismic studies have provided significant understanding of the ongoing crustal deformation in the Current Tectonic Regime (CTR) on a regional scale^{11–13} in the Himalaya. However, the site-specific studies are few^{3,6,7–9,14–16}. Identification of active faults that have moved within the CTR, i.e. during Holocene, also helps in assessing whether or not tectonic movements are likely to occur and cause seismicity, generally associated with these faults.

In the Outer Himalaya, lying between the Himalayan Frontal Thrust (HFT) and Main Boundary Thrust (MBT) in the north, numerous active faults and neotectonic features have been reported^{2,3,15,17–19}, which have generated major and great earthquakes^{20–22}. We have carried out a study of these features in an active segment of the Outer Himalaya between the Satluj and Ghaggar rivers and referred to as the Pinjaur Dun (Figure 1), which lies in the meizoseismal zone of the 1905 Kangra earthquake²³. While the authors and their co-workers have already recorded active faults in the Pinjaur–Kalka area and near Chandigarh^{8,9,16}, the present communication reports the discovery of a few more active structures. We have identified ten such faults. However, we discuss here only five major fault systems, since the analysis of other structures is in progress and shall be communicated separately in the near future.

The multi-spectral satellite data of IRS-ID-LISS-III and PAN (date: 04 October 2002) and air photos, supported by the Survey of India Topographic Maps (1:50,000 scale), constituted the main data source for the present study. The satellite data have been digitally enhanced for feature extraction. Selected ground-truth checks have been carried out to re-judge the interpretation.

The Pinjaur Dun is one of the three major Duns in the western Frontal Himalaya, viz. Soan, Pinjaur and Dehra. The Duns are broad synclinal depressions which develop when the growing outer ridges constituted by the Siwalik sediments block and divert the drainage^{11,24}. The Pinjaur Dun is NW–SE trending, approximately 55 km long and 12 km at its widest part. It is bound by the Siwalik Hills in the southwest and by the Kasauli–Ramshahr ranges in the northeast. This Dun closes in the east near Malla where the Siwalik and the eastern extension of Kasauli–Ramshahr ranges approach each other. It extends westwards across the River Satluj (Figures 1 and 2). Sirsa is the main axial

*For correspondence. (e-mail: gphilip@wihg.res.in)