4
Results

4.1. Survey of ethnomedicinal herbs of Darjeeling hills

The survey revealed that all the 106 herbs collected are commonly used by the local people for treating various ailments. Amongst the various parts of the plants, aerial ones (70%) are more extensively used than the underground ones (30%), for treating about 40 different types of ailments (Table 5), *viz.* cut/ wound, pneumonia, fever, diarrhoea, dysentery, food poisoning, fracture, tuberculosis etc. It was also evident that among the tribes of Darjeeling hills, herbs (65%) are preferred medicinal source than the shrubs (18%), and climbers (17%).

4.2. Screening of plants

Of 123 plants collected, 38 herbs/herbal parts were selected for the study of antioxidant and antimicrobial activities on the basis of their anticipated nontoxicity, since majority of the plant parts are consumed by the local people.

4.3. *In vitro* antioxidant activity screening

For preliminary screening, the DPPH$^{\bullet}$ assay for free radical-scavenging, reducing power and iron (II) chelating potential of the test samples were carried out to judge their relative antioxidant activity.

Table 5. Collection of medicinally important plants/ plant parts from Darjeeling hills

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
11.05.05	*Adhatoda vasica* Nees in Wall.	basak	Acanthaceae	s	leaf	cough, cold	chewed in empty stomach	5985
11.05.05	*Costus speciosus* (Koen.) J.E.Smith.	betlouri	Costaceae	s	root	jaundice	fresh roots are chewed for 10 days	5983
11.05.05	*Drymaria cordata* (L.) Willd.	abijalo	Caryophyllaceae	h	leaf	sinusitis, pnuemonia	juice is consumed twice	5991
11.05.05	*Elsholtzia blanda* (Benth.)	mrigay-jhar/ mirey-jhar	Lamiaceae	h	leaf	diarrhoea	filtered juice is consumed twice for 2 days	5999
11.05.05	*Eupatorium glandulosum* Kunth.	banmara	Asteraceae	s	leaf	cut, wound	paste is applied	5987
11.05.05	*Houttuynia cordata* Thunburgh.	gandey-jhar	Saururaceae	h	leaf	tuberculosis	decoction (2 tsp) taken for 15 days	5984
11.05.05	*Hydrocotyle himalaica* P.K. Mukherjee	botuke-jhar	Apiaceae	h	leaf	tonsillitis	decoction is used for gargle	5990
11.05.05	*Ocimum sanctum* L.	babariphool	Lamiaceae	h	leaf	fever and cold	juice is mixed with ginger and consumed at bed time	5986
11.05.05	*Pouzolzia hirta* (Blume) Hassk.	chipley	Urticaceae	h	root	bone fracture	paste is bandaged around the affected area	6026
11.05.05	*Solanum nigrum* L.	khursane-jhar	Solanaceae	h	aerial part	sedative	juice is consumed	5993
11.05.05	*Stephania rotunda* sensu Hook.f. et Thoms.	seto-tamarke	Menispermaceae	c	root	diabetes	decoction is consumed in empty stomach	6001
18.05.05	*Asparagus racemosus* Willd.	satamuli	Asparagaceae	h	root	diabetes	powder is consumed	6004
18.05.05	*Astilbe rivularis* D. Don.	bansupari	Saxifragaceae	h	root	bleeding after childbirth	dried and chewed	6015

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
18.05.05	*Cardamine hirsuta* L.	simrayo	Brassicaceae	h	aerial part	low pressure, in cardiac problems	cooked and consumed	6082
18.05.05	*Clematis buchananiana* Wall.	pinase lahara	Ranunculaceae	c	leaf, root	sinusitis	burnt and its smoke inhaled	6005
18.05.05	*Duchesnea indica* (Andrews) Focke.	thare oonew	Rosaceae	h	leaf	cut, wound	paste is applied externally	5999
18.05.05	*Fragaria daltoniana* J.Gay	bhui-ka-phal	Rosaceae	h	root	toothache	smoked as cigar	
18.05.05	*Heracleum nepalense* D. Don	chimping	Apiaceae	h	seed	fever, influenza	dried seeds are chewed	6014
18.05.05	*Lycopodium* sp.	nagbeli	Lycopodiaceae	h	spore	bleeding after childbirth	powdered spores are consumed	6008
18.05.05	*Potentilla kleiniana* Wright & Arn.	kauwa ka phal	Rosaceae	h	root	burn	root pasted with coconut oil is applied externally	6006
18.05.05	*Rubia manjith* Roxb. Ex Fleming.	magito	Rubiaceae	c	root	skin disease	paste applied externally	6010
18.05.05	*Rubus ellipticus* Smith.	ainselu	Rosaceae	s	fruit	gastritis	rind is consumed	6003
18.05.05	*Solanum khasianum* Clarke.	beek ara	Solanaceae	h	thorn	toothache	powder is put in the affected tooth	6002
18.05.05	*Zanthoxylum acanthopodium* DC.	boke timboor	Rutaceae	s	fruit	indigestion	eaten	6007
19.06.05	*Bergenia ciliata* (Hwarth) Sternberg.	pakhanbet	Acanthaceae	h	leaf	dysentery	paste is consumed	6029
19.06.05	*Diplazium esculentum* (Retz.) SW.ex Schrad	ningro	Woodsiaceae	h	frond	constipation, maintains good stomach condition	eaten as curry	

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
19.06.05	*Equisetum debile* Roxb. Ex Vaucher.	kurkure jhar	Equisetaceae	h	aerial part	mouth sore	paste applied externally	6020
19.06.05	*Hypericum patulum* Thunb.	urilo	Clusiaceae	s	bark	cut, wound	paste applied externally	6022
19.06.05	*Lygodium flexnosum* (L.) Sw.	bahun lahara	Schizaeaceae	h	young frond	rheumatism, sprain	Fronds soaked in mustard oil and externally applied on affected areas	6035
19.06.05	*Stephania elegans* Hook. f. & Thomson.	tamarke	Menispermaceae	c	root	diabetes	soaked overnight in water and the water is drunk	
19.06.05	*Tectaria coadunata* (J. Sm.) C.Chr.	kali oonew	Aspidaceae	h	whole plant	dysentery	boiled and taken as soup	6091
19.06.05	*Tinospora cordifolia* (Willd.) Miers.	gurjo-lahara	Memispermaceae	c	fruit	tuberculosis	boiled in milk and drunk for 10-15 days	6064
02.10.05	*Abrus pulchellus* L.	lalgeri	Fabaceae	c	fresh root	hill dysentery	filtered juice is consumed	6058
02.10.05	*Acorus calamus* L.	bojho	Acoraceae	h	rhizome	osteoarthritis	crushed, boiled with salt and the decoction is massaged	6051
02.10.05	*Desmodium triflorum* (L.) DC.	sano-charmeli	Fabaceae	h	leaf	diarrhoea, dysentery	filtered juice is consumed	6096
02.10.05	*Geranium sp.*	ragatgeri	Geraniaceae	h	whole plant	renal disease, dysentery	filtered juice is consumed	5997
02.10.05	*Girardinia diversifolia* (Link).	bhangre-sisnu	Urticaceae	s	root, inflorescence	bone fracture, high pressure, diabetes	paste eaten	6090
02.10.05	*Gloriosa superba* L.	bikh-phool	Colchicaceae	h	leaf	rheumatism, skin disease, leprosy, piles	paste is used for external application	

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
02.10.05	*Hyptis suaveolens* L.Poit	gange-jhar	Lamiaceae	h	leaf	lice, parasitic infestations	juice is warmed and applied	6070
02.10.05	*Mimosa pudica* L.	buari-jhar	Mimosaceae	s	root	toothache	powder is used for tooth cleaning	6057
02.10.05	*Osbeckia nepalensis* Hooker.	angeri	Melastomataceae	s	young leaf or tender shoot	pneumonia, fever, common cold	extract is applied on forehead and chest	6053
02.10.05	*Sida acuta* Burm. f.	khareto	Malvaceae	s	stem	bone fracture	paste is applied externally	6042
22.07.06	*Achyranthes bidentata* Blume.	ankhlay-jhar	Amaranthaceae	h	root	rheumatism, gout	juice with salt massaged	6040
22.07.06	*Acmella calva* (DC.) Jansen.	kalijhar	Asteraceae	h	flower, inflorescence	toothache, decay, mouth sore	chewed to relieve pain	6066
22.07.06	*Ageratum conyzoides* L.	jiamae-jhar	Asteraceae	h	fresh leaf or young shoot	cut, injuries for blood clotting, sores	paste applied externally	6038
22.07.06	*Canna indica* L.	phool tarul	Cannaceae	h	root	gonorrhoea	paste applied on the genitals	6068
22.07.06	*Cannabis sativa* L.	ghanja / gagina	Cannabaceae	h	root	indigestion, acidity	chewed	6032
22.07.06	*Cynodon dactylon* (L.) Pers.	dubo	Poaceae	h	aerial part	nose bleeding, cut, indigestion, body swelling	juice is used	
22.07.06	*Dioscorea tuberosa* Vell.	gittha	Dioscoreaceae	c	tuber	gastritis	filtered juice is consumed	6047
22.07.06	*Eupatorium odoratum* L.	banmara	Asteraceae	s	leaf	cut, wound	paste applied externally	6086

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
22.07.06	*Kaempferia rotunda* L.	bhuichampa	Zingiberaceae	h	root	bone fracture, joint dislocation, sprain, gout, rheumatism	paste is bandaged in soft cloth	
22.07.06	*Mussaenda roxburghii* Hook.f.	dhobinikath	Rubiaceae	s	root	jaundice	filtered juice is consumed orally	6049
22.07.06	*Pouzolzia indica* (L.) Wight.	chipley	Urticaceae	h	root	bone fracture	plastered with *P. hirta* and *Curcuma longa*	6095
22.07.06	*Scoparia dulcis* L.	chinijhar	Scrophulariaceaa	h	leaf	diabetes	eaten	
02.09.06	*Abroma augustum* (L.) L.f.	shringraj	Sterculiaceae	s	young shoot	discharges in females	decoction is consumed, in which shoots are soaked overnight	6037
02.09.06	*Flemingia strobilifera* Roxb.	barkaulijhar	Leguminosae	s	root	indigestion, insomnia, epilepsy	juice 2 teaspoon twice	6080
02.09.06	*Oxalis corniculata* L.	chariamilo	Oxalidaceae	h	leaf	eye infection	filtered juice is applied	
02.09.06	*Paederia foetida* L.	biri-lahara	Rubiaceae	c	leaf	diarrhoea, dysentery	cooked and eaten	6062
02.09.06	*Persicaria capitata* (D. Don) h. Gross.	ratnowlo	Polygonaceae	h	leaf	insect sting	paste applied externally	6019
02.09.06	*Physalis minima* L.	phak-phakay	Solanaceae	h	leaf	earache, boils, diuretic	juice is applied externally	
02.09.06	*Plantago erosa* Wall. In Roxb.	jibre-jhar	Plantaginaceae	h	seed	dysentery	juice is consumed	
02.09.06	*Pupalia atropurpurea* Moq. In DC.	ulta-kuro	Amaranthaceae	h	leaf	dysentery tonsillitis	decoction is consumed gargled	6033

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
02.09.06	*Solanum torvum* Swartz.	ban-bihi	Solanaceae	s	leaf	toothaches	rolled into cigars and smocked	6036
02.09.06	*Sonchus arvensis* auct.non.L.	ban-rayo	Asteraceae	h	root	toothache	paste is applied on affected tooth	6023
02.09.06	*Trichosanthes lepiniana* Naudin.	indraynee	Cucurbitaceae	c	mature fruit	diabetes	dry powder is consumed after lunch	6039
02.09.06	*Tupistra nutans* Wall. ex Lindl.	nakima	Convallariaceae	h	inflorescence	food-poisoning	boiled, cooked and consumed	6089
27.01.07	*Leucas indica* (L.) Sm.	dulphe jhar	Lamiaceae	h	leaf , flower	rheumatism, headache, common cold, sores	burnt and the ash is smelled	
02.09.06	*Urtica ardens* Blume.	sisnu	Urticaceae	h	root	kidney stones	juice is consumed	6018
06.05.07	*Amaranthus spinosus* L.	lonre	Amaranthaceae	h	leaf	burns, boils, as laxative	eaten as curry	6094
06.05.07	*Amaranthus viridis* L.	lude	Amaranthaceae	h	leaf	stomach colic, as laxative	filtered juice is consumed with sugar	6000
06.05.07	*Andrographis paniculata* (Burm.f.) Wallich.	kalmegh	Acanthaceae	h	leaf	constipation, for blood purification	made pills and consumed in empty stomach in morning	6073
06.05.07	*Bauhinia vahlii* wight and Arn.	malu	Leguminosae	c	bark	dysentery	filtered juice is consumed	6040
06.05.07	*Blumea hieracifolia* (Don) D.G.	sahasrabooti	Asteraceae	h	leaf	asthma	dried and smelled	6075
06.05.07	*Cissus quadrangularis* L.	harhjorha	Vitaceae	c	whole plant	bone fracture	paste applied externally	6083
06.05.07	*Cyperus rotundus* L.	mothe	Cyperaceae	h	root	analgesic, sedative	decoction is consumed	6055

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
06.05.07	*Euphorbia adenophorum* Sprengel	kalo banmara	Asteraceae	h	leaf	external injuries, cut	paste applied externally	6084
06.05.07	*Euphorbia sikkimensis* Boiss.	dudhe	Euphorbiaceae	h	root	boils	applied externally	5989
06.05.07	*Mentha arvensis* L.	padina	Lamiaceae	h	leaf	digestion	chewed	6030
06.05.07	*Piper longum* L.	pipla	Piperaceae	c	fruit	cold, cough	fried and eaten	
06.05.07	*Piper nigrum* L.	pipla	Piperaceae	c	fruit	prolonged cough	milled with ginger and honey, and consumed	6078
17.06.07	*Calamintha umbrosa* (M.Bieb.) Fisch & Mey. Ind.Sem. Hort.Petrop.	bilajor	Lamiaceae	h	whole plant	good health	eaten as vegetable to ensure good health	6016
17.06.07	*Centella asiatica* (L.) Urban.	golpatta	Apiaceae	h	leaf	cough	washed and juice is consumed	6012
17.06.07	*Chenopodium album* L.	bethe	Chenopodiaceae	h	leaf	gastritis, body pain	consumed as soup	6100
17.06.07	*Clerodendrum viscosum* Vantenat.	bhat	Lamiaceae	s	leaf	leucoderma, hydrophobia	juice is consumed	6034
17.06.07	*Coccinia* sp.	kundri	Cucurbitaceae	c	fruit	diabetes	eaten raw	6087
17.06.07	*Curcuma longa* L.	hardi	Zingiberaceae	h	root	cough, cold, as an antiseptic in sores and wounds	decoction is consumed	6081
17.06.07	*Cymbopogon pendulus* (Nees ex Steud.) Will. Watson	kagati ghas	Poaceae	h	whole plant	fever	filtered juice is consumed	6092
17.06.07	*Drymaria diandra* (Blume)	pothe	Caryophyllaceae	h	leaf	headache, throat pain,	juice is consumed	6067

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
17.06.07	*Enhydra fluctuens* Loureiro.	hincha	Asteraceae	h	leaf	skin disease, liver problem, diabetes, bronchitis	aqueous extract is used	
17.06.07	*Euphorbia hirta* L.	ratulo	Euphorbiaceae	h	latex	warts, cut	applied externally	6046
17.06.07	*Hedyotis corymbosa* (L.) Lam.	bakhri lahara	Rubiaceae	s	root	stomach colic, gastritis, food poisoning	juice is drunk thrice a day till recovery	6063
17.06.07	*Luffa aegyptiaca* Miller	ghyura	Cucurbitaceae	c	fruit	stomach disorder	pulp is consumed	6052
17.06.07	*Melastoma malabathricum* L.	chulasi	Melastomataceae	s	stem bark, root	wounds, skin diseases	paste applied externally	6065
17.06.07	*Physalis peruviana* L.	jangli mewa	Solanaceae	s	leaf	fever, pneumonia, cold	paste is used on neck, forehead	
17.06.07	*Phytolacca acinosa* Roxburgh.	jaringo	Phytolaccaceae	h	leaf	high blood pressure	juice is consumed	6025
17.06.07	*Pratia nummularia* Benth. ex Kurz.	lanka-sanay	Campanulaceae	h	leaf and	dysentery, tonsillitis	juice is used	6098
					root	snakebite	paste	
01.07.07	*Fragaria nubicola* Lindl.	bhui-ainselu	Rosaceae	h	root	cough, cold, toothache, anticonvulsive, high altitude sickness	juice 4 teaspoon twice a day	6103
					fruit	digestive and laxative	ripe fruit is chewed	

Date of collection	Scientific name	Local name	Family	Habit[a]	Part used	Disease treated	Mode of administration	CIMAP[b] Accn No.
01.07.07	*Panax pseudo-ginseng* N. Wallich.	panch-patey	Araliaceae	h	root	liver disorder, stomach colic, antipyretic, menstrual disorder	juice is consumed (2 tsp thrice a day for 7 days)	6102
25.08.07	*Vitex negundo* L.	simali	Lamiaceae	c	stem	body- swelling, common cold and influenza and bone fracture	steam bath paste is bandaged	6045
25.08.07	*Passiflora foetida* L.	Sano jhar	Passifloraceae	c	leaf	insomia, hysteria and epilepsy and as painkiller	infusion of leaves are consumed	6079
25.08.07	*Commelina benghalensis* L.	kane jhar	Commelinaceae	h	leaf	conjunctivitis	applied 2 drops for 4 days on eyes	6041
25.08.07	*Nephrolepis cordifolia* (L.)Presl.	pani amala	Davalliaceae	h	tuber	burning urination, diabetes, high blood pressure	extract is consumed	
25.08.07	*Ocimum tenuiflorum* L.	babari-phool	Lamiaceae	h	inflorescence	influenza, cold, asthma, bronchitis	chewed in empty stomach	
25.08.07	*Plantago major* L.	chamche-jhar	Plantaginaceae	h	leaf, flower and fruit	throat pain, cut, wounds	juice in consumed in case of throat pain and flower and fruit juice are applied externally	6099
25.08.07	*Pteris* sp.	oonew	Pteridaceae	h	frond	dysentery	consumed	6088
25.08.07	*Stephania hernandiifolia* Walp.	panhelo tamarke	Menispermaceae	c	tuber	diabetes	chewed	6093

[a]s, shrub; h, herb; c, climber
[b]CIMAP, Central Institute of Medicinal and Aromatic Plants, Lucknow, India

4.3.1. DPPH•-scavenging

The assay was carried out with a fixed concentration of all the extracts (1 mg ml^{-1}). *F. nubicola* root (FNR), *P. hirta, E. fluctuens, O. tenuiflorum, Pteris* sp., *C. buchananiana, P. major, C. album, D. indica* and *G. superba* showed excellent DPPH•-scavenging ability (>80%), while the activities of *S. hernandifolia, L. indica, A. rivularis* and *C. rotundus* were good (60-76%) (Table 6). Amongst these, the extracts of FNR, *P. hirta* and *E. fluctuens* showed comparable activity as that (96%) of 1 mg butylated hydroxyanisole (BHA) ml^{-1}. Interestingly, *F. nubicola* fruit extract showed only moderate (43%) DPPH•-scavenging.

Table 6. Preliminary antioxidant parameters of the plant extracts[a]

Plant	Antioxidant parameter			SA[e]
	DPPH[b]	RP[c]	MCA[d]	
Acmella calva	46.3 ± 2.32[1]	553.7 ± 24.81[38]	10.0 ± 0.41[10,13]	m
Amaranthus spinosus	24.2 ± 2.14[2]	164.3 ± 12.42[37]	51.0 ± 0.61[12]	p
Amaranthus viridis	5.0 ± 0.34[3]	121.3 ± 9.27[36]	48.7 ± 0.52[12]	p
Astilbe rivularis	66.8 ± 3.48[4]	762.3 ± 9.77[35]	2.0 ± 0.11[3,5]	g
Cardamine hirsuta	12.8 ± 1.21[5,8]	118.0 ± 8.24[34,36]	29.3 ± 0.44[7,11]	p
Chenopodium album	86.6 ± 1.44[6]	418.1 ± 4.80[33]	94.8 ± 7.50[6]	e
Clematis buchananiana leaf	87.9 ± 6.22[6,7]	202.7 ± 5.92[32]	10.6 ± 0.62[10]	e
Clematis buchananiana roots	11.3 ± 0.80[5]	39.7 ± 3.52[31]	17.1 ± 0.91[1]	p
Cyandon dactylon	16.6 ± 1.17[8]	22.7 ± 1.88[30,31]	7.2 ± 0.24[4,5,13]	p
Cyperus rotundus	60.7 ± 2.24[9]	394.1 ± 4.51[29]	4.3 ± 1.42[3,4,5]	g
Desmodium triflorum	23.6 ± 1.77[2]	16.9 ± 0.92[28,30]	8.5 ± 0.38[4,10]	p
Diplazium esculentum	25.4 ± 2.11[2]	112.0 ± 7.40[34,36]	58.8 ± 3.08[9]	p
Duchesnea indica	84.8 ± 0.86[6,10]	251.1 ± 11.55[26]	43.1 ± 3.12[8]	e
Enhydra fluctuens	91.5 ± 0.51[7,11]	822.5 ± 5.27[25]	72.4 ± 4.66[6]	e
Equisetum debile	15.0 ± 0.92[8]	60.7 ± 4.80[24]	40.1 ± 2.50[8]	p
Fragaria nubicola fruit	43.0 ± 3.79[1,12]	477.0 ± 22.56[23]	7.5 ± 0.92[4,5,10]	m
Fragaria nubicola root	92.3 ± 5.32[11]	927.0 ± 11.04[22]	25.0 ± 1.18[7]	e
Gloriosa superba	81.4 ± 2.25[10,13]	388.1 ± 3.08[21]	2.5 ± 0.11[3,5]	e
Heracleum nepalense	34.0 ± 2.48[14]	502.3 ± 34.11[20]	14.1 ± 0.25[1]	m
Hottuynia cordata	45.3 ± 3.74[1]	719.0 ± 32.87[19]	10.6 ± 0.64[4,10]	m
Leucas indica	77.2 ± 2.46[3,13]	327.2 ± 2.28[18]	9.8 ± 0.56[4,10]	g
Nephrolepis cordifolia	2.88 ± 0.09[15]	9.6 ± 0.85[17,28,30]	1.6 ± 0.08[3,5]	p
Ocimum tenuiflorum	90.8 ± 0.74[6,11]	868.1 ± 7.40[16]	73.0 ± 5.21[6]	e
Paederia foetida	46.6 ± 5.28[1]	489.6 ± 22.50[20,23]	0.6 ± 0.01[3,5]	m
Panax pseudoginseng	5.8 ± 0.31[3,15,16]	10.9 ± 0.32[14,17,28,30]	32.3 ± 2.45[2,11]	p
Perilla fructescence	21.3 ± 1.68[2]	288.7 ± 15.55[13]	7.9 ± 0.92[4,5,10]	p
Physalis minima	29.9 ± 1.52[14]	22.8 ± 1.28[12,14,17,28,30,31]	92.8 ± 2.11[6]	p
Physalis peruviana	39.7 ± 2.71[12]	27.0 ± 2.80[12,14,17,28,30,31]	2.9 ± 0.28[3,5]	m
Plantago major	87.1 ± 1.68[6]	297.0 ± 5.66[10,13]	6.2 ± 0.35[4]	e
Pouzolzia hirta	92.0 ± 2.11[7,11]	409.0 ± 2.73[29,33]	4.9 ± 0.32[3,4,5]	e
Pouzolzia indica	26.8 ±1.50[2,14,17]	80.7 ± 5.23[8]	4.9 ± 0.45[3,4,5]	p
Pratia numularia	9.9 ± 0.62[5,16]	19.0 ± 0.60[7,11,12,14,17,28,30]	27.5 ± 1.12[7]	p
Pteris sp.	89.1 ± 4.50[6,11]	300.3 ± 17.36[10,13]	6.6 ± 0.38[4]	e
Rubia manjith	37.2 ± 2.42[12,14]	82.3 ± 4.55[8]	6.7 ± 0.32[4,13]	m
Sonchus arvensis	24.6 ± 1.18[2,17]	26.9 ± 2.61[4,7,11,12,14,17,28,30,31]	2.8 ± 0.32[3]	p
Stephania hernandifolia	77.4 ± 6.10[13]	165.2 ± 11.41[37]	32.8 ± 2.23[2]	g
Tectaria coadunata	9.9 ± 0.28[5,16]	45.0 ± 2.21[4,11,24,31]	31.4 ± 2.36[2,11]	p
Tupistra nutans	11.8 ± 0.76[5]	141.0 ± 7.82[1]	16.8 ± 0.58[1]	p

[a]Values are mean with standard error of measurements (n = 3).
[b]% scavenging of DPPH• by the test extracts (1 mg ml^{-1}) after 30 min incubation.
[c]mg ascorbic acid equivalent (AAE) g^{-1} dry weight of the extract.
[d]% inhibition of Fe (II)-ferrozine complex formation by the test extracts (1 mg ml^{-1}).
[e]SA, scavenging activity; e, excellent (≥ 80%); g, good (60-<80%); m, moderate (30-<60%); p, poor (<30%).
Values of means with same superscript in any given column are same at $P<0.05$.

Based on DPPH•-scavenging results, the test extracts were categorized into four broad classes, *viz.* excellent, good, moderate and poor radical-scavengers (Table 6). It is well-known that DPPH• is a *N*-centred stable free radical, resembling the biologically relevant lipid peroxyl radical. DPPH•-scavenging activity

often correlates with the ability of the test sample with its anti-lipid peroxidation (LPO) activity. Hence, for the categorization of the antioxidant property of the 38 extracts, emphasis was given on their DPPH•-scavenging ability.

4.3.2. Reducing power (RP)

The respective RP values (Table 6) of the test samples revealed that FNR, *O. tenuiflorum*, and *E. fluctuens* were the most potent followed by *A. rivularis* that also showed good DPPH•-scavenging activity. The correlation between reducing power and DPPH•-scavenging activity was found to be quite high, in most of the categories.

4.3.3. MC power

Besides oxygen metabolism, various reactive oxygen species (ROS) can be formed in cells by the transition metals, especially Fe(II)-mediated reactions and radiation exposure leading to deleterious effects on membrane lipids and DNA. Endogenously available metal ions are known to initiate and propagate LPO chain process via decomposition of the initially formed lipid hydroperoxides (Braughler *et al.*, 1987). Therefore, Fe(II)-chelating ability is an added advantage for antioxidants. Amongst the plants in the excellent category, significant MC was found with *C. album* > *O. tenuiflorum* ~ *E. fluctuens* > *D. indica* > FNR. In the good category, *S. hernandifolia* showed better MC property, while *P. foetida* (moderate class) was almost inactive (Table 6). Surprisingly, some of the plants with poor DPPH•-scavenging ability showed a significant MC property. Amongst the test samples, *C. album* showing moderate DPPH•-scavenging was the best Fe(II)-chelator (95%), better than EDTA (1 mg ml^{-1}) that showed 70% chelation under identical conditions.

4.4. Phenolic and flavonoid contents

The plant phenolics act as defense against various stresses and often account for the antioxidant and antimicrobial activities of the plants. In addition, the class of phenolics, known as flavonoids, is also credited with antioxidant activity and various other health benefits. Hence, the total phenolic and flavonoid contents (designated as TPC and TFC, respectively) of the extracts were also determined and the results are presented in Table 7. The relative order of the plants with high TPC was *H. cordata* >> *E. debile* > *Pteris* sp. > *C. buchananiana* leaf. Likewise, the plants, *Pteris* sp., FNR, *P. peruviana*, *S. hernandifolia* and *P. foetida* showed impressive TFC values. Notably, *Pteris* sp. scored high in both the assays, while FNR was enriched with flavonoids that might account for its excellent DPPH•-scavenging property. Grossly, the pteridophytes were found to contain high phenolics. Although *C. album* and *G. superba* contained a very low amount of phenol and flavonoids, those exhibited a very good antioxidant activity.

4.5. Principal Component Analysis (PCA) of antioxidant parameters

To understand the interrelationships among the four measured antioxidant parameters *viz.* DPPH•-scavenging, iron chelation, and RP with TPC plus TFC of the plant extracts, PCA was performed. The loadings of variables in the analyses are summarized in Table 8, while Fig. 4 depicting the PCA plot provides a clear picture of the correlation of the PC with the original variables and between antioxidant activity parameters.

A factor rotation using the Varimax method was performed and three factor loadings were obtained that accounted for the 81% of the total variance of the plant extracts, chosen on the basis of their eigen values (>1). The loading in the PCA plot expresses the correlation of the PC with the original variables and between antioxidant parameters with TPC and TFC. TFC, DPPH•-scavenging ability and RP were shown to be highly loaded on factor 1 (PC1) with loadings 0.555, 0.929 and 0.795, respectively. DPPH•-scavenging

Table 7. Total phenolic and flavonoid contents of the lyophilized extracts[a]

Plant	TPC[b]	TFC[c]
Acmella calva	137.5 ± 5.4[24]	21.6 ± 0.7[33]
Amaranthus spinosus	103.5 ± 8.1[23]	7.3 ± 0.2[32]
Amaranthus viridis	82.6 ± 1.3[22]	6.8 ± 0.06[31,32]
Astilbe rivularis	26.6 ± 2.2[21]	16.2 ± 1.4[30]
Cardamine hirsuta	78.8 ± 0.6[22]	10.0 ± 0.06[29,31,32]
Chenopodium album	8.6 ± 0.6[20]	7.5 ± 0.5[28,29,31,32]
Clematis buchananiana leaf	472.4 ± 11.7[19]	51.7 ± 0.8[13]
Clematis buchananiana roots	108.6 ± 8.7[18,23,24]	7.3 ± 0.03[28,29,31,32]
Cyandon dactylon	44.1 ± 3.4[17]	4.2 ± 0.3[27,28,31,32]
Cyperus rotundus	107.4 ± 3.1[16,23]	14.1 ± 2.9[26,29,30]
Desmodium triflorum	21.1 ± 1.7[15,21]	10.9 ± 0.9[25,25,28,29,31,32]
Diplazium esculentum	82.5 ± 4.9[14]	19.0 ± 1.6[24,30,33]
Duchesnea indica	31.5 ± 1.9[13,21]	28.3 ± 1.6[23]
Enhydra fluctuens	16.2 ± 0.3[11,15,19]	14.1 ± 0.4[22,25,26,29,30]
Equisetum debile	532.5 ± 3.9[12]	33.9 ± 0.9[21]
Fragaria nubicola fruit	38.1 ± 2.1[1,13,17]	9.3 ± 0.5[20,25,28,29,31,32]
Fragaria nubicola root	130.1 ± 9.4[5]	106.7 ± 8.8[19]
Gloriosa superba	13.0 ± 0.8[11,19]	10.2 ± 0.7[18,22,25,26,28,29,31,32]
Heracleum nepalense	38.7 ± 0.1[1,17]	24.8 ± 0.1[17,23,33]
Hottuynia cordata	2275.5 ±1.6[10]	28.1 ± 0.9[16,17,23]
Leucas indica	26.9 ± 2.1[9,13,15,21]	25.4 ± 1.5[16,17,23,33]
Nephrolepis cordifolia	32.5 ± 0.08[1,8,9,13,21]	0.8 ± 0.02[15,27]
Ocimum tenuiflorum	26.4 ± 2.4[4,8,9,13,15,21]	25.0 ± 1.8[14,16,17,23,33]
Paederia foetida	125.9 ± 10.2[5]	49.9 ± 3.6[13]
Panax pseudoginseng	16.6 ± 1.1[4,7,11,15]	2.9 ± 0.1[12,15,27,31]
Perilla fructescence	18.9 ± 0.3[4,11,15,21]	0.1 ± 0.01[11,12,15,27]
Physalis minima	16.6 ± 1.8[4,7,11,15]	8.4 ± 0.4[10,18,20,25,27,28,29,31,32]
Physalis peruviana	164.1 ± 5.8[8]	96.0 ± 1.2[9]
Plantago major	19.9 ± 2.2[4,7,9,11,15,21]	14.4 ± 1.1[8,18,22,25,26]
Pouzolzia hirta	35.0 ± 2.6[1,13]	21.0 ± 1.6[7,14,17,24,33]
Pouzolzia indica	14.4 ± 0.6[7,11,15,19]	10.9 ± 1.4[2,8,10,18,20,22,25,26,28,29,31]
Pratia numularia	13.4 ± 1.5[7,11,15,19]	13.1 ± 1.6[2,8,18,20,22,25,26,29,30]
Pteris sp.	502.2 ± 4.8[6]	178.3 ± 9.7[6]
Rubia manjith	107.5 ± 9.5[5,16]	18.2 ± 1.2[5,7,8,22,24,26,30,33]
Sonchus arvensis	24.2 ± 1.4[4,9,13,15,21]	23.9 ± 2.1[4,7,14,16,17,33]
Stephania hernandifolia	175.6 ± 8.5[3]	86.1 ± 5.5[3]
Tectaria coadunata	258.8 ± 9.1[2]	11.0 ± 0.8[2,8,10,18,20,22,25,26,28,29,31,32]
Tupistra nutans	34.3 ± 1.0[1,9,13,21]	6.5 ± 0.1[1,10,12,18,20,27,28,29,31,32]

[a]Values are mean with standard error of measurements (n = 3).
[b]mg GAE g^{-1} dry weight of the extract.
[c]mg ECE g^{-1} dry weight of the extract.
Values of means with same superscript in any given column are same at $P<0.05$.

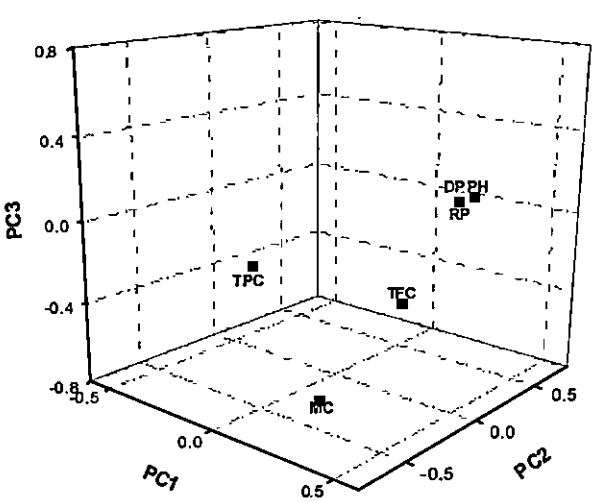


Fig. 4. Loading plot of the different antioxidant parameters and phenol and flavonoid contents of the 38 herbs, analysed using principal component analysis.

ability and RP were closely loaded, which indicated the two properties are intimately related to the antioxidant activity. However, the high loading (-0.978) of TPC on factor 3 (PC3) where other parameters have low loading indicated its independence from the other variables. On the other hand, the Fe(II)-chelating ability of the plant extracts shows a loading of -0.853, while TFC has a loading of 0.622 on Factor 2 (PC2), which showed low loading of the other parameters.

Table 8. Loadings of variables (antioxidative parameters and phenol and flavonoid contents) by individual principal components (PCs)

Variable	Loading			Communality
	PC1	PC2	PC3	
TPC	0.058	0.115	-0.978	0.973
TFC	0.555	0.622	-0.081	0.701
DPPH	0.929	0.128	0.119	0.893
RP	0.795	-0.212	-0.250	0.740
MCA	0.135	-0.853	0.084	0.753

4.6. Further antioxidant screening

The extracts, showing good to excellent DPPH•-scavenging ability (Table 6) were chosen for further evaluation of their antioxidant activity. To this end, their ABTS•+ and hydroxyl radical (•OH)-scavenging activity as well as oxygen radical absorption capacity (ORAC) were assessed. The collective results are shown in Table 9.

Table 9. Antioxidant activities of lyophilized extracts of some selected plants[a]

Plant	ABTS•+ scavenging (%)	ORAC (mg GAE g^{-1} extract)	•OH scavenging (%)
Astilbe rivularis	42.6 ± 4.3**	509.8 ± 6.14	33.9 ± 3.1$
Chenopodium album	60.4 ± 3.1**	ND	67.5 ± 1.4$
Clematis buchananiana leaf	59.8 ± 5.6**	919.4 ± 8.52#	0
Cyperus rotundus	59.5 ± 3.5**	515.1 ± 5.53	72.7 ±2.5$
Duchesnea indica	52.4 ± 2.5**	506.7 ± 6.89	54.8 ± 2.7$
Enhydra fluctuens	63.2 ± 2.7*	263.1 ± 8.28#	32.1 ± 2.1$
Fragaria nubicola root	66.43 ± 6.1	1387.6 ± 11.1#	85.4 ± 2.4$
Gloriosa superba	55.3 ± 2.9**	409.9 ± 4.68#	49.1 ± 4.5$
Leucas indica	51.4 ± 3.1**	ND	59.1 ± 3.6$
Ocimum tenuiflorum	61.7 ± 2.9*	352.3 ± 5.7#	11.5 ± 0.8$
Plantago major	57.7 ± 3.4**	ND	60.9 ± 3.5$
Pouzolzia hirta	64.8 ± 4.5	ND	29.1 ± 1.9$
Pteris sp.	60.5 ± 6.6**	606.9 ± 9.57#	46.5 ± 3.3$
Stephania hernandifolia	60.8 ± 6.2**	547.6 ± 4.56#	52.6 ± 2.4$

[a]All the assays were carried out using 1 mg ml^{-1} of test samples. Values are mean with standard error of measurements (n = 3).
*$P < 0.05$, compared to BHA.
**$P < 0.01$, compared to BHA.
$$P<0.01$, compared to mannitol.
#Values are different with each other at $P<0.01$.

4.6.1. ABTS•+-scavenging ability

Except for *A. rivularis* extract that showed 43% scavenging, other test samples showed similar and good activity 60-67% in this assay (Table 9).

4.6.2. •OH-scavenging activity

The extracts of FNR and *C. rotundus* showed excellent •OH-scavenging (85% and 73%, respectively), while *P. major*, *C. album*, *D. indica*, *S. hernandifolia* and *L. indica* showed 55-60% activity. *C. buchananiana* leaf extract was inactive and *O. tenuiflorum* showed marginal activity (Table 9). Although *P. hirta*, *E. fluctuens* and *A. rivularis* showed moderate activity, the phenol content and preliminary antioxidant screening were found to be powerful.

4.6.3. ORAC

The ORAC assay gives a measure of the capacity of a test sample to scavenge peroxyl radicals, generated by spontaneous decomposition of AAPH. This assay has been successfully used to assess the antioxidant property of a wide variety of edible plant extracts containing a diverse array of phytochemicals such as

alkaloids, coumarins, flavonoids, phenylpropanoids, terpenoids and phenolics acids (Aruoma, 2003; Domínguez *et al.*, 2005). Hence, ORAC values of the plant extracts were estimated in terms of gallic acid equivalents. In this assay, FNR extract showed a superior capacity, and the extracts of *C. buchananiana* leaf, *Pteris* sp., *D. indica*, *S. hernandifolia*, *A. rivularis*, and *C. rotundus* were found potent (Table 9).

4.6.4. Protection against LPO

LPO, which often results by the Fenton-mediated process, is one of the most damaging events in free radicals-mediated cellular injury. Hence, the protective activity of a fixed concentration (1 mg ml^{-1}) of the test samples against iron-mediated LPO was examined, and the results are summarized in Table 10. Amongst the test samples, *P. major* showed insignificant protection against LPO, while *E. fluctuens* showed moderate protection (34%). The activities of the other extracts were impressive. FNR, *G. superba* and *S. hernandifolia* showed equivalent potency as that of BHA. Although the results with FNR and *G. superba* correlated well with their DPPH•-scavenging activities, the poor protection offered by *E. fluctuens*, an excellent DPPH• scavenger, was unexpected.

Table 10. Protective activity of selected plant extracts against lipid peroxidation[a]

Treatment	Malonaldialdehyde[b]	% protection[c]
experimental control	49.9 ± 5.3	0
Astilbe rivularis	14.1 ± 1.1	71.74***
Chenopodium album	13.5 ± 0.9	72.95***
Clematis buchananiana leaf	14.4 ± 1.7	71.14***
Cyperus rotundus	12.3 ± 0.8	75.35***
Duchesnea indica	9.6 ± 1.5	80.76***
Enhydra fluctuens	32.9 ± 3.5	34.07*
Fragaria nubicola root	5.2 ± 0.6	89.66***
Gloriosa superba	5.1 ± 0.3	89.78***
Leucas indica	13.6 ± 1.2	72.75***
Ocimum tenuiflorum	9.7 ± 0.7	80.56***
Plantago major	46.5 ± 3.8	6.81
Pouzolzia hirta	17.2 ± 2.1	65.53**
Pteris sp.	21.2 ± 2.5	57.52**
Stephania hernandifolia	7.2 ± 0.9	85.57***
BHA	4.5 ± 0.6	90.98***

[a]All the assays were carried out using 1 mg ml^{-1} of test samples. Values are mean with standard error of measurements (n = 3).
[b]nmol MDA mg^{-1} protein.
[c]% reduction of MDA formation with respect to the experimental control.
*$P<0.05$, compared to BHA.
**$P<0.01$, compared to BHA.
***$P<0.001$, compared to BHA.

4.7. Comprehensive analyses of the antioxidant capacity of FNR

The above results clearly revealed that the extract of FNR possessed the best antioxidant activity. Hence, a comprehensive analysis of its antioxidant property was carried out using several *in vitro* methods. In addition, its antiproliferative activity was also investigated.

4.7.1. $ABTS^{•+}$-scavenging

FNR and BHA (positive control) showed a dose-dependent scavenging of the $ABTS^{•+}$ with the IC_{50} values of 276 µg ml^{-1} and 187 µg ml^{-1}, respectively (Fig. 5). The percentage of scavenging increased sharply up to 250 µg ml^{-1} of both the samples, beyond which the increase was gradual. At the lower concentration range (up to 250 µg ml^{-1}), this corresponds exactly ($P < 0.01$) with the activity of BHA.

4.7.2. DPPH•-scavenging

FNR and BHA also showed a concentration-dependent DPPH•-scavenging activity throughout the entire range of test concentrations (up to 100 µg ml^{-1}). From this, the respective IC_{50} values of FNR and BHA for DPPH•-scavenging were found as 49.5 and 45.7 µg ml^{-1}, respectively (Fig. 6). In separate experiments, It was also found that the reactions with $ABTS^{•+}$ and DPPH• were quite fast and almost in all cases complete in 1-3 min and ~15 min, respectively (data not shown).

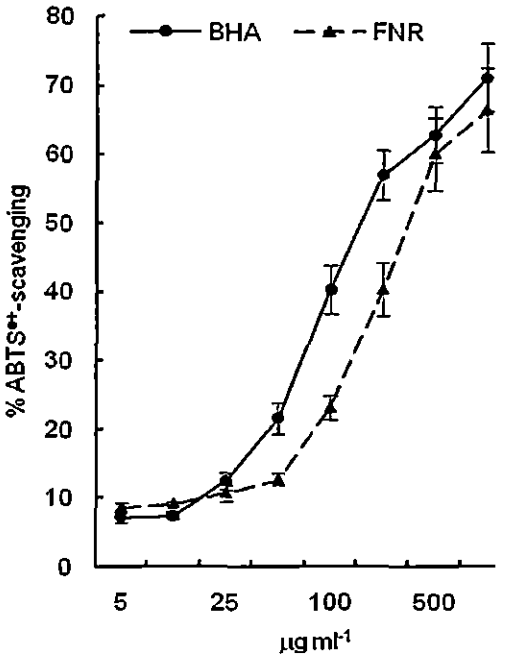


Fig. 5. $ABTS^{\bullet+}$-scavenging potential of *Fragaria nubicola* root extract (FNR) and butylated hydroxyanisole (BHA). The values are mean ± SEM (n = 3).

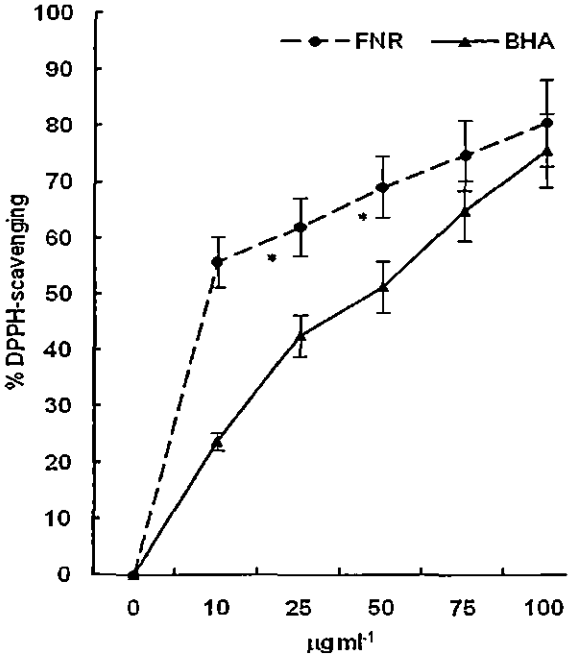


Fig. 6. $DPPH^{\bullet}$-scavenging potential of *Fragaria nubicola* root extract (FNR) and butylated hydroxyanisole (BHA). The values are mean ± SEM (n = 3). $^{*}P<0.01$, compared to BHA.

4.7.3. RP

The reducing power of FNR (final concentration, 10-100 μg ml^{-1}), was measured in terms of mg AAE g^{-1} lyophilized extract, determined from its iron(III)-reducing potential which also showed a concentration-dependent increase (Fig. 7).

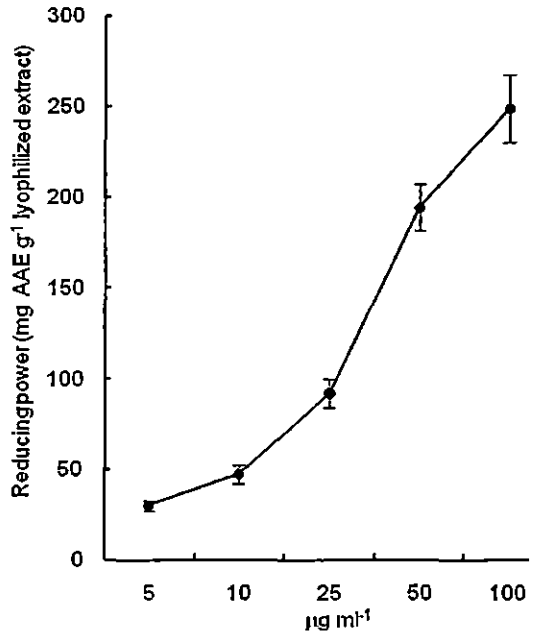


Fig. 7. Reducing power of *Fragaria nubicola* root extract. The values are mean ± SEM (n = 3). The values at 10-100 μg ml^{-1} were significantly different ($P<0.01$).

4.7.4. Anti-LPO

The protective capacity of FNR against LPO was assessed using mice liver mitochondria as a convenient lipid source, and the Fenton reagent (Fe(II)/ascorbic acid) or AAPH as the radical initiator. The end product of LPO was measured in terms of malondialdehyde (MDA).

In unstimulated experiments, the amount of MDA in mice liver mitochondria was marginal, which increased to 22.7 nmol mg^{-1} protein on stimulation with Fe(II)/ascorbic acid. FNR inhibited LPO in a concentration-dependent manner (Fig. 8), with an IC_{50} value of 10.1 μg ml^{-1}. Under similar conditions, 1 μM BHA (positive control) provided 50.2% protection against LPO (not shown in the graph). In the AAPH-induced LPO, the protective activity of FNR was significantly less ($P < 0.05$) with an IC_{50} value of 13.8 μg ml^{-1}.

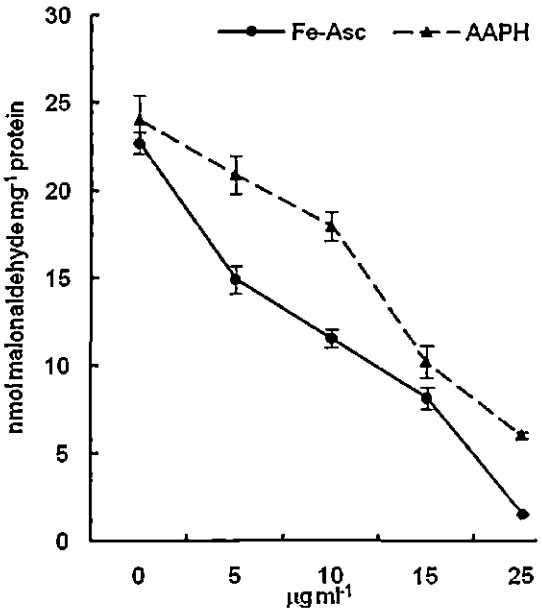


Fig. 8. Protective activity of *Fragaria nubicola* root extract (FNR) against Fe(II)-ascorbic acid (Fe-Asc) and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation. The values are mean ± SEM (n = 3). The concentrations of FNR, reducing the malonyldehyde concentration by 50% (IC_{50}) compared to the experimental control were calculated from the graph. The IC_{50} values of FNR in the AAPH or Fe-Asc-induced lipid peroxidation were significantly different ($P<0.05$).

4.7.5. DNA protection

The protective effect of FNR against Fenton-induced damage to pBR322 DNA was studied by separating the different DNA bands by electrophoresis. A representative gel electrophoresis photograph showing the protective activity of FNR is presented in Fig. 9. The quantitative protection offered by FNR revealed its remarkable protecting ability even at very low concentrations (Table 11). For example, 25 µg FNR ml^{-1} extract showed 94.9% protection against the Fenton-induced DNA nicking.

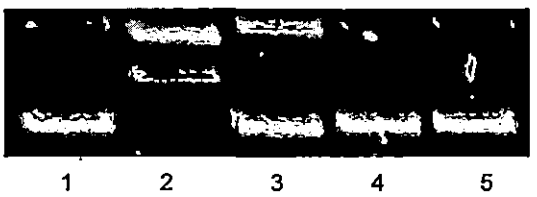


Fig. 9. Concentration dependent protective activity of *Fragaria nubicola* root extract (FNR) against Fenton-mediated DNA nicking as revealed by DNA gel electrophoresis. Lane 1, native DNA; Lane 2, DNA + Fenton reagent; Lanes 3-5, DNA + Fenton reagent + FNR (0.5, 2.5 and 5 µg ml^{-1}, respectively).

Table 11. Concentration-dependent protective activity of *Fragaria nubicola* root (FNR) against Fenton-mediated damage to plasmid DNA[a]

Sample	% of supercoiled DNA	% of open-circular DNA	% of linear DNA	% protection
Native DNA	94.93	5.07	0	100
DNA + Fenton reagent (A)	0	14.4	85.6	0
A + FNR (0.5 µg ml^{-1})	40.5	59.5	0	62.7
A + FNR (2.5 µg ml^{-1})	91.2	8.8	0	96.1
A + FNR (5 µg ml^{-1})	92.91	7.09	0	97.9

[a]Values are mean of duplicate sets.

4.7.6. Radioprotection of proteins

For this assay, γ-rays were used for the oxidation of bovine serum albumin (BSA) as a model protein. The protective activity of FNR was examined by comparing the fluorescence of unirradiated protein samples *vis-à-vis* that of those, irradiated in the absence and presence of FNR (2.5-10 µg ml^{-1}) and the positive control, Trolox (2.5 µM).

Exposure of BSA to γ-rays at a dose of 300 Gy reduced the protein fluorescence by 76%. Addition of FNR to BSA, prior to γ-irradiation, increased the protection dose-dependently, although at 2.5 µg ml^{-1}, it was inactive (Table 12). Under similar conditions, Trolox, a water-soluble analogue of vitamin E, did not show any protective effect.

4.8. Toxicity of FNR

Toxicity of various concentrations of FNR to the normal cells was assessed by the methyl tetrazolium salt (MTT) using INT-407 as the model system. As revealed by the MTT results (Fig. 10), FNR was nontoxic to

Table 12. Concentration-dependent protective activity of *Fragaria nubicola* root (FNR) against γ-ray-mediated oxidation of bovine serum albumin (BSA)[a]

Treatment	fluorescent unit	% protection
BSA (unirradiated)	2.16 ± 0.16	100
γ-irradiated BSA (A)	0.52 ± 0.07*	0
A + FNR (2.5 μg ml^{-1})	0.54 ± 0.05	1.2
A + FNR (5 μg ml^{-1})	1.22 ± 0.09**	42.5
A + FNR (10 μg ml^{-1})	1.54 ± 0.14**	62.6
A + Trolox (2.5 μM)	0.52 ± 0.06	0

[a]BSA was γ-irradiated at 25°C at a dose of 300 Gy (dose rate, 16 Gy min^{-1}) and the fluorescence of the samples was estimated at λ_{ex} = 280 nm and λ_{em} = 345 nm. Values are presented as mean with standard error of measurements (n = 3). The % protection was calculated from the reduction of fluorescence intensity with respect to irradiated control.
*P<0.001, compared to unirradiated sample.
**P<0.001, compared to irradiated sample.

antiproliferative capacity towards both the cell lines. Although the effect was dose-dependent, the sensitivities towards the cell lines were different. Thus while its toxicity towards the A-549 cells increased monotonously, its efficacy against the MCF-7 cells was comparatively less at the higher concentration range. Under identical conditions, the IC_{50}-values of the positive control, curcumin, against A-549 was 26.7 μM (data not shown). The nontoxicity of the vehicle (0.1% DMSO) against the cell lines was also confirmed.

4.10. HPLC of FNR

HPLC analysis of acid hydrolysate of FNR showed the presence of 11 compounds. The peaks were detected at the λ_{max} (280 nm) of FNR (Fig. 11). Based on comparison of the HPLC profile with those of standards, seven of the constituents were identified as ascorbic acid and six hydrolyzable tannins as shown in Table 13. FNR was found to be rich in ascorbic acid, gallic acid, and dihydrocaffeic acid, besides containing small amounts of ellagic acid, genistic acid, caffeic acid and *o*-coumaric acid.

the INT-407 cells up to 200 μg ml^{-1} after incubation for 72 h. Hence, it promises to be a good antioxidant for further investigation in cellular systems.

4.9. Antiproliferative activity of FNR

Cytotoxic effect of FNR at various concentrations on two different human cancer cell lines *viz.* lung (A-549) and breast (MCF-7) cells was also examined. The MTT assay results at 48 h (Fig. 10) established its pronounced toxicity against both the cell lines. Even at low concentrations (upto 25 μg ml^{-1}), FNR showed good

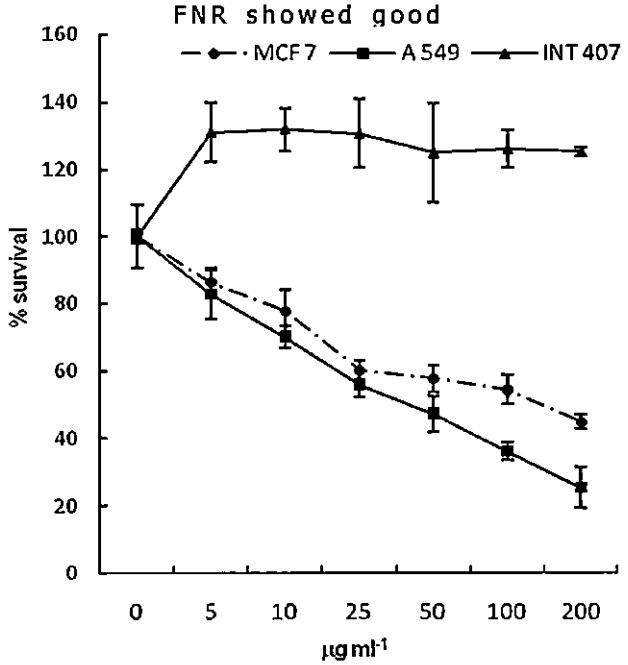


Fig. 10. Influence of *Fragaria nubicola* root extract against normal (INT-407) and lung (A549) and breast (MCF-7) cancer cell lines. Values (mean ± SEM; n = 18) are expressed in percentage considering that of the untreated control cells as 100. In normal cell line values were not significantly different, compared to control cells. In cancerous cell lines, the IC_{50} values were significantly different (P<0.01).

4.11. Antimicrobial assay of plant extracts

Disc diffusion method is extensively used to assay the antimicrobial activity of natural substances and plant extracts. The assay is based on the use of discs as reservoirs containing solutions of substances to be examined (Gülcin *et al.*, 2003). Using the assay, the antimicrobial activity of 38 extracts of 36 different herbs was examined. For this, three Gram-negative bacteria (*E. coli*, *K. pnuemoniae* and *S. typhi*), and six

Table 13. Chemical composition[a] of *Fragaria nubicola* root extract, as revealed in HPLC analysis (vide Fig. 11)

Peak no.	Compound	Retention time (min)	Quantity (μg mg^{-1} extract)
1	unidentified	2.88	nd[b]
2	ascorbic acid	2.99	97.9 ± 3.45
3	unidentified	3.18	nd
4	unidentified	3.52	nd
5	gallic acid	4.36	51.4 ± 2.61
6	unidentified	5.03	nd
7	genistic acid	5.48	1.9 ± 0.16
8	dihydrocaffeic acid	6.44	11.3 ± 1.42
9	ellagic acid	8.36	5.3 ± 0.67
10	caffeic acid	9.05	4.4 ± 0.32
11	*o*-coumaric acid	12.30	1.1 ± 0.15

[a]Values are mean with standard error of measurements
[b]nd, not determined.

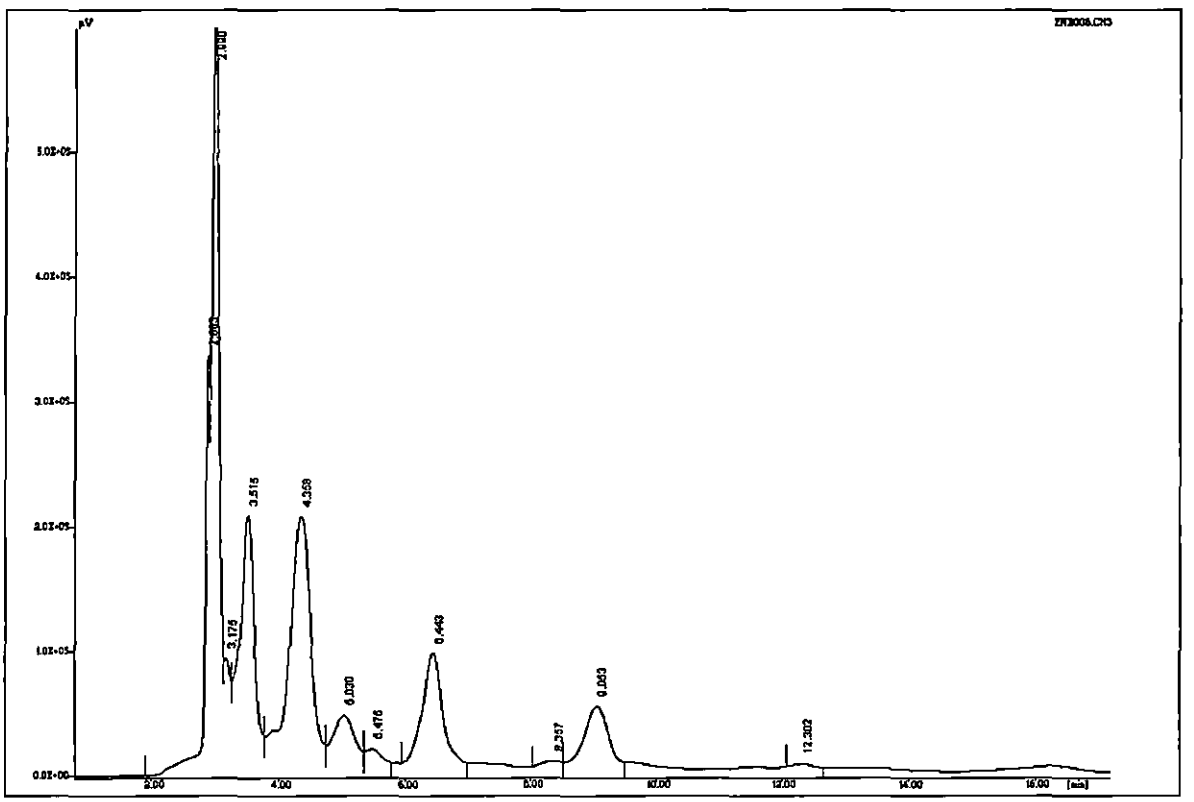


Fig. 11. HPLC profile of *Fragaria nubicola* root extract. Numerical values represent the peak numbers, mentioned in Table 13.

Gram-positive bacteria (*B. subtilis*, *B. cereus*, *B. pumilus*, *B. licheniformis*, *L. monocytogenes* and *S. aureus*), two yeasts (*C. albicans* and *S. cerevisiae*) and two moulds (*A. niger* and *A. alternata*) were chosen as the target organisms. The results of the studies are shown in Tables 14-16.

Thirty of the tested extracts were active against most of the Gram-positive bacteria. Especially, the extracts of *D. indica*, FNR, *L. indica*, *P. minima*, *P. peruviana*, *Pteris* sp. and *S. hernandifolia* inhibited all the test bacteria in this category, and many of these showed very low MIC values (<1 mg ml^{-1}). The extracts were not very active against the Gram-negative bacteria tested; the most potent ones are *R. manjith* which showed inhibitory activity against *K. pnuemoniae*, *A. rivularis* and *D. indica* which showed inhibitory activity against *S. typhi*, and *A. viridis* which showed inhibitory activity against *E. coli* (Table 14). On the other hand, *Physalis* extracts were active against Gram-positive bacteria (Table 15), but did not

Table 14. Antimicrobial activities of the herbs against Gram-negative bacteri

Plant	MIC (mg disc^{-1})		
	Escherichia coli	*Klebsiella pnuemoniae*	*Salmonella typhi*
Acmella calva	>8.0	>8.0	4.0-5.0
Amaranthus spinosus	7.0-8.0	>8.0	>8.0
Amaranthus viridis	2.0-3.0	>8.0	4.0-5.0
Astilbe rivularis	>8.0	>8.0	1.0-2.0
Cardamine hirsuta	>8.0	>8.0	>8.0
Chenopodium album	>8.0	>8.0	>8.0
Clematis buchananiana leaf	2.0-3.0	>8.0	7.0-8.0
Clematis buchananiana root	>8.0	>8.0	7.0-8.0
Cyandon dactylon	>8.0	>8.0	>8.0
Cyperus rotundus	>8.0	>8.0	7.0-8.0
Desmodium triflorum	>8.0	6.0-7.0	>8.0
Diplazium esculentum	>8.0	>8.0	>8.0
Duchesnea indica	5.0-6.0	6.0-7.0	1.0-2.0
Enhydra fluctuens	>8.0	>8.0	>8.0
Equisetum debile	>8.0	7.0-8.0	>8.0
Fragaria nubicola fruit	6.0-7.0	1.0-2.0	6.0-7.0
Fragaria nubicola root	>8.0	6.0-7.0	4.0-5.0
Gloriosa superba	3.0-4.0	>8.0	>8.0
Heracleum nepalense	>8.0	>8.0	>8.0
Hottuynia cordata	6.0-7.0	>8.0	>8.0
Leucas indica	>8.0	>8.0	>8.0
Nephrolepis cordifolia	>8.0	>8.0	>8.0
Ocimum tenuiflorum	7.0-8.0	>8.0	>8.0
Paederia foetida	>8.0	>8.0	6.0-7.0
Panax pseudoginseng	6.0-7.0	>8.0	>8.0
Perilla fructescence	>8.0	>8.0	>8.0
Physalis minima	>8.0	>8.0	6.0-7.0
Physalis peruviana	7.0-8.0	>8.0	>8.0
Plantago major	>8.0	>8.0	>8.0
Pouzolzia hirta	>8.0	>8.0	>8.0
Pouzolzia indica	>8.0	>8.0	>8.0
Pratia numularia	>8.0	>8.0	>8.0
Pteris sp.	>8.0	>8.0	6.0-7.0
Rubia manjith	>2.0	1.0-2.0	>2.0
Sonchus arvensis	>8.0	>8.0	>8.0
Stephania hernandifolia	>8.0	7.0-8.0	5.0-6.0
Tectaria coadunata	>8.0	>8.0	5.0-6.0
Tupistra nutans	>8.0	7.0-8.0	>8.0

show appreciable activity against the Gram-negative ones. *Listeria monocytogenes*, an important foodborne pathogen, was strongly inhibited by *S. hernandifolia*, *H. nepalense*, *E. fluctuens* and *R. manjith*. The results revealed that the MIC values of the plant extracts were much lower for the Gram-positive bacteria, compared to the Gram-negative ones.

The yeasts, *C. albicans* and *S. cerevisiae*, were inhibited by eight extracts; *Candida albicans*, a potential human pathogen, was best inhibited by *H. nepalense* and *T. nutans* (Table 16). The moulds were quite resistant to the extract. *A. alternata* was inhibited by 45% of the extracts, while *A. niger* was susceptible to 24% only of the extracts used. *H. nepalense* and *T. nutans* could inhibit the growth of all the tested organisms at 2-5 mg ml^{-1} and 0.5-4 mg ml^{-1}, respectively.

4.12. Isolation of active principles from extracts

4.12.1. Furanocoumarins from *H. nepalense* fruits

Besides showing moderate antioxidant activity, the methanol extract of *H. nepalense* fruit possessed significant activity against various bacteria, yeasts and moulds. Hence, the phytochemical investigation was pursued to isolate and identify some of its major components.

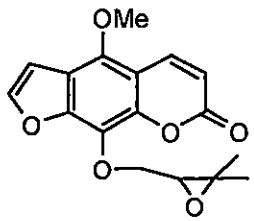
Earlier, several furanocoumarins have been isolated from *Heracleum* spp. (Banerjee *et al.*, 1980; Kavli *et al.*, 1983; Sajjadi and Noroozi, 2007; Tosun *et al.*, 2008). Due to the wide occurrence of coumarins, including furanocoumarins in plants, and their diverse biological activities (Borges *et al.*, 2005; Fylaktakidou *et al.*, 2004; Kulkarni *et al.*, 2006), they are valued in medicinal chemistry. Considering their high solubility in nonpolar organic solvents, the 1.4 g-hexane extract (10% w w^{-1}) of *H. nepalense* fruits was subjected to column chromatography over silica gel, and the three fractions eluting with 15-25% ethyl acetate/ hexane furnished three furanocoumarins after PTLC.

These were identified as byakangelicol (BA), sphondin (SD) (Tosun *et al.*, 2008)) and furopinnarin (FP) (Banerjee *et al.*, 1980) from their respective ^{1}H NMR spectrum that matched with reported values.

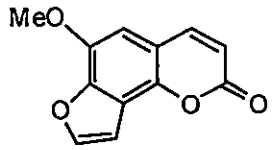
Table 15. Antimicrobial activities of the herbs against Gram-positive bacteria

Plant	MIC (mg disc^{-1})					
	Bacillus subtilis	*Bacillus cereus*	*Bacillus pumilus*	*Bacillus licheniformis*	*Listeria monocytogenes*	*Staphylococcus aureus*
Acmella calva	7.0-8.0	5.0-6.0	>8.0	4.0-5.0	3.0-4.0	>8.0
Amaranthus spinosus	>8.0	6.0-7.0	>8.0	>8.0	>8.0	7.0-8.0
Amaranthus viridis	>8.0	5.0-6.0	>8.0	4.0-5.0	4.0-5.0	6.0-7.0
Astilbe rivularis	0.5-1.0	1.0-2.0	7.0-8.0	7.0-8.0	2.0-3.0	1.0-2.0
Cardamine hirsuta	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Chenopodium album	7.0-8.0	3.0-4.0	>8.0	>8.0	>8.0	>8.0
Clematis buchananiana leaf	1.0-2.0	>8.0	>8.0	2.0-3.0	5.0-6.0	0.5-1.0
Clematis buchananiana root	0.5-1.0	7.0-8.0	>8.0	4.0-5.0	3.0-4.0	1.0-2.0
Cyandon dactylon	>8.0	7.0-8.0	>8.0	>8.0	>8.0	>8.0
Cyperus rotundus	6.0-7.0	3.0-4.0	>8.0	7.0-8.0	>8.0	2.0-3.0
Desmodium triflorum	4.0-5.0	3.0-4.0	0.25-0.5	>8.0	>8.0	0.5-1.0
Diplazium esculentum	6.0-7.0	>8.0	>8.0	>8.0	>8.0	5.0-6.0
Duchesnea indica	3.0-4.0	1.0-2.0	0.125-0.25	0.5-1.0	>8.0	0.125-0.25
Enhydra fluctuens	1.0-2.0	>8.0	0.5-1.0	0.25-0.5	1.0-2.0	0.25-0.5
Equisetum debile	7.0-8.0	7.0-8.0	>8.0	>8.0	>8.0	>8.0
Fragaria nubicola fruit	7.0-8.0	6.0-7.0	5.0-6.0	>8.0	>8.0	1.0-2.0
Fragaria nubicola root	0.5-1.0	0.25-0.5	0.125-0.25	0.5 -1.0	5.0-6.0	<0.125
Gloriosa superba	6.0-7.0	7.0-8.0	6.0-7.0	2.0-3.0	>8.0	1.0-2.0
Heracleum nepalense	1.0-2.0	3.0-4.0	2.0-3.0	6.0-7.0	2.0-3.0	2.0-3.0
Hottuynia cordata	6.0-7.0	>8.0	4.0-5.0	>8.0	4.0-5.0	>8.0
Leucas indica	0.125-0.25	0.5-1.0	0.25-0.5	0.25-0.5	7.0-8.0	0.25-0.5
Nephrolepis cordifolia	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
Ocimum tenuiflorum	6.0-7.0	2.0-3.0	1.0-2.0	1.0-2.0	5.0-6.0	0.5-1.0
Paederia foetida	>8.0	>8.0	>8.0	>8.0	7.0-8.0	>8.0
Panax pseudoginseng	7.0-8.0	7.0-8.0	2.0-3.0	7.0-8.0	>8.0	0.125-0.25
Perilla fructescence	>8.0	>8.0	>8.0	>8.0	>8.0	7.0-8.0
Physalis minima	0.5-1.0	>8.0	0.25-0.5	0.125-0.25	4.0-5.0	0.125-0.25
Physalis peruviana	0.125-0.25	0.5-1.0	0.5-1.0	0.25-0.5	>8.0	2.0-3.0
Plantago major	7.0-8.0	1.0-2.0	1.0-2.0	5.0-6.0	>8.0	6.0-7.0
Pouzolzia hirta	5.0-6.0	3.0-4.0	>8.0	>8.0	>8.0	0.5-1.0
Pouzolzia indica	>8.0	6.0-7.0	7.0-8.0	>8.0	>8.0	6.0-7.0
Pratia numularia	7.0-8.0	>8.0	>8.0	>8.0	2.0-3.0	0.125-0.25
Pteris sp.	0.25-0.5	0.5-1.0	1.0-2.0	0.5-1.0	>8.0	0.25-0.5
Rubia manjith	1.0-2.0	>2.0	>2.0	<0.125	0.125-0.5	0.125-0.25
Sonchus arvensis	>8.0	>8.0	>8.0	7.0-8.0	>8.0	3.0-4.0
Stephania hernandifolia	0.125-0.25	0.125.25	0.5-1.0	0.25-0.5	0.5-1.0	0.5-1.0
Tectaria coadunata	0.5-1.0	5.0-6.0	6.0-7.0	>8.0	>8.0	>8.0
Tupistra nutans	>8.0	>8.0	2.0-3.0	>8.0	>8.0	6.0-7.0

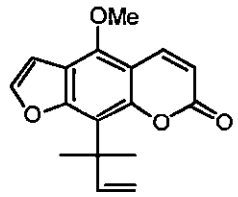
Amongst these, BA and SD were earlier isolated from *Heracleum crenatifolium* fruits (Tosun *et al.*, 2008), while FP was obtained from *Heracleum thomsoni* roots (Banerjee *et al.*, 1980). The ^{1}H-NMR spectral data of the compounds are presented below, while their chemical structures are shown in Fig. 12.



Byak-angelicol



Sphondin



Furopinnarin

Fig. 12. Chemical structures of the furanocoumarins, isolated from *Heracleum nepalense*

The ^{1}H NMR spectrum (Fig. 13) of BA showed three 3-protons singlets at δ 1.21, 1.29 and 4.15 for the *gem*-dimethyl and methoxy groups, along with a 1H-triplet at δ 3.26 (J = 5.6 Hz, epoxy proton) and a 2H-doublet at δ 4.39 (J = 5.6 Hz, -CH_2O). The pair of 1H-doublets at δ 6.23 and δ 8.08, each with

Table 16. Antimicrobial activities of the herbs against yeasts and moulds

Plant	MIC (mg disc^{-1})			
	Candida albicans	*Saccharomyces cerevisiae*	*Aspergillus niger*	*Alternaria alternata*
Acmella calva	7.0-8.0	>8.0	>8.0	3.0-4.0
Amaranthus spinosus	>8.0	>8.0	>8.0	4.0-5.0
Amaranthus viridis	>8.0	>8.0	>8.0	>8.0
Astilbe rivularis	>8.0	>8.0	>8.0	1.0-2.0
Cardamine hirsuta	>8.0	>8.0	1.0-2.0	7.0-8.0
Chenopodium album	7.0-8.0	5.0-6.0	>8.0	>8.0
Clematis buchananiana leaf	>8.0	>8.0	>8.0	1.0-2.0
Clematis buchananiana root	>8.0	>8.0	4.0-5.0	7.0-8.0
Cyandon dactylon	>8.0	>8.0	>8.0	>8.0
Cyperus rotundus	>8.0	>8.0	>8.0	5.0-6.0
Desmodium triflorum	>8.0	>8.0	>8.0	>8.0
Diplazium esculentum	>8.0	>8.0	2.0-3.0	4.0-5.0
Duchesnea indica	>8.0	>8.0	>8.0	>8.0
Enhydra fluctuens	>8.0	>8.0	>8.0	>8.0
Equisetum debile	>8.0	>8.0	2.0-3.0	6.0-7.0
Fragaria nubicola fruit	>8.0	>8.0	0.5-1.0	0.25-0.5
Fragaria nubicola root	>8.0	>8.0	3.0-4.0	2.0-3.0
Gloriosa superba	>8.0	>8.0	>8.0	>8.0
Heracleum nepalense	4.0-5.0	4.0-5.0	2.0-3.0	4.0-5.0
Hottuynia cordata	5.0-6.0	7.0-8.0	>8.0	3.0-4.0
Leucas indica	6.0-7.0	7.0-8.0	>8.0	>8.0
Nephrolepis cordifolia	>8.0	>8.0	>8.0	>8.0
Ocimum tenuiflorum	5.0-6.0	5.0-6.0	>8.0	>8.0
Paederia foetida	>8.0	>8.0	>8.0	>8.0
Panax pseudoginseng	>8.0	>8.0	>8.0	>8.0
Perilla fructescence	>8.0	>8.0	1.0-2.0	>8.0
Physalis minima	>8.0	>8.0	>8.0	>8.0
Physalis peruviana	7.0-8.0	>8.0	>8.0	>8.0
Plantago major	>8.0	5.0-6.0	>8.0	>8.0
Pouzolzia hirta	>8.0	>8.0	>8.0	0.5-1.0
Pouzolzia indica	>8.0	>8.0	>8.0	1.0-2.0
Pratia numularia	>8.0	>8.0	>8.0	>8.0
Pteris sp.	>8.0	>8.0	>8.0	>8.0
Rubia manjith	>2.0	>2.0	>2.0	>2.0
Sonchus arvensis	>8.0	>8.0	>8.0	>8.0
Stephania hernandifolia	>8.0	6.0-7.0	>8.0	>8.0
Tectaria coadunata	>8.0	>8.0	>8.0	4.0-5.0
Tupistra nutans	0.5-1.0	1.0-2.0	0.5-1.0	3.0-4.0

coupling constant 9.8 Hz accounted for the coumarin double bond, while another pair of 1H-doublets at δ 6.98 and δ 7.59 with a low *J*-value (2.4 Hz) establised its disubstituted furan moiety.

For SD, similar ^{1}H NMR resonances for the furanocoumarin moiety were present. The additional 1-proton singlet at the aromatic region (δ 6.77) and the absence of the more up field signals established that one of carbons of the coumarin phenyl group was unsubstituted by the epoxyhydroxymethyl group as in BA.

On the other hand, the ^{1}H NMR spectral features of furocoumarin moiety in furopinnarin (FP) were similar to that of BA. However, the pair of ^{1}H NMR resonances at δ 5.58 (t, 1H) and δ 4.82 (d, 2H) established the presence of a terminal olefin group, while that of a *gem*-dimethyl group was inferred from the two 3-protons singlets at δ 1.68 and 1.71.

9-((3,3-Dimethyloxiran-2-yl)methoxy)-4-methoxy-7*H*-furo[3,2-g]chromen-7-one [(±)-byakangelicol]: Yield: 29.5 mg (0.21%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08 (d, *J* = 9.8 Hz, 1H), 7.59 (d, *J* = 2.4 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.23 (d, *J* = 9.8 Hz, 1H), 4.39 (d, *J* = 5.6 Hz, 2H), 3.26 (t, *J* = 5.6 Hz, 1H), 4.15 (s, 3H), 1.29 (s, 3H), 1.21 (s, 3H).

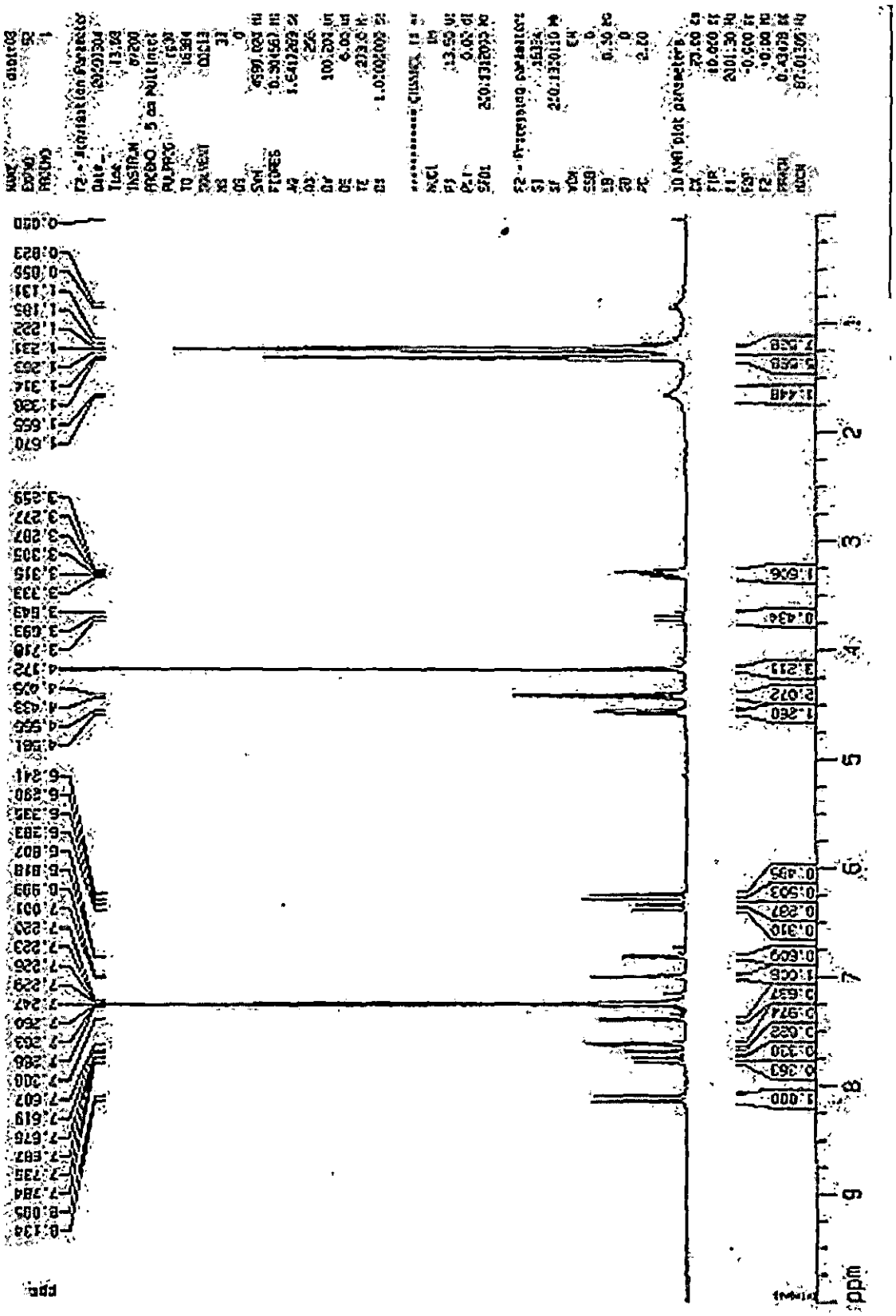


Fig. 13. ^{1}H NMR spectrum of byak-angelicol

6-Methoxy-2*H*-furo[2,3-*h*]chromen-2-one (sphondin): Yield: 10.5 mg (0.075%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.75 (d, *J* = 9.6 Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 6.99 (d, *J* = 2.2 Hz, 1H), 6.77 (s, 1H), 6.39 (d, *J* = 9.6 Hz, 1H), 3.93 (s, 3H).

4-Methoxy 9-(2-methylbut-3-en-2-yl)-7*H*-furo[3,2-g]chromen-7-one (furopinnarin): Yield: 15.6 mg (0.11%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.09 (d, *J* = 9.8 Hz, 1H), 7.60 (d, *J* = 2.3 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 9.8 Hz, 1H), 5.58 (t, *J* = 7.2 Hz, 1H), 4.82 (d, *J* = 7.2 Hz, 2H), 4.15 (s, 3H), 1.71 (s, 3H), 1.68 (s, 3H).

4.12.2. Alkaloids from *S. hernandifolia* roots

The root extract of *S. hernandifolia* also showed a good antioxidant activity, as well as an excellent antibacterial activity against all Gram-positive and Gram-negative (except *E. coli*) bacteria. Especially, its activity against the Gram-positive bacterium *L. monocytogenes* was exceptionally good. Hence, a preliminary phytochemical investigation of the extract was carried out. The extract was found to be enriched with several alkaloids as revealed by the Dragendorff test. From the acid soluble portion of the extract, a mixture of alkaloids was isolated. However, due to the close R_f of the constituents, the compounds could not be purified.

4.12.3. Antioxidant and antimicrobial activities of *H. nepalense* furanocoumarins

4.12.3.1. DPPH•-scavenging

None of the furanocoumarins showed significant DPPH•-scavenging activity. Amongst these, BA was marginally (15%) active at 0.2 mM, which did not improve at the higher concentrations. SD and FP, on the other hand, showed 21% scavenging at 1 mM.

4.12.3.2. Anti-LPO

All the compounds showed a dose-dependent protection against Fenton-mediated LPO. BA and SP showed a better protection, while the anti-LPO of FP was manifested at the higher concentrations.

4.12.3.3. •OH-scavenging

Amongst the furanocoumarins, FP showed some (25%) •OH-scavenging activity only at 0.8 mM. The other two compounds scavenged the radical dose-dependently. BA was the most active amongst the tested furanocoumarins. The entire antioxidant data are summarized in Table 17.

4.12.3.4. DNA protection

Exposure of pBR322 plasmid DNA to Fenton reagent led to the formation of open circular and linear forms. BA restored the supercoiled (SC) form up to 5 μg ml^{-1}, but led to drastic DNA damage beyond 5 μg ml^{-1}, as revealed from the reduction of the SC form (data not shown).

On the other hand, FP restored the SC form dose-dependently, as revealed from the increase in the SC form that was reduced (80.6%) drastically on exposure to the Fenton reagent. The results are summarized in Fig. 14 and 15 and Tables 18 and 19.

4.12.4. Antimicrobial activity of isolated furanocoumarins

All the compounds showed moderate antibacterial activity against the Gram-positive and Gram-negative bacteria. BA showed better inhibition (100 μM) against all the tested bacteria. Interestingly, they were also

Table 17. Concentration-dependent DPPH·-scavenging, Fenton-mediated lipid peroxidation inhibitory and hydroxyl radical-scavenging activities of the isolated byak-angelicol (BA), sphondin (SD) and furopinnarin (FP)[a]

Furanocoumarin	%DPPH·-scavenging[a]			nmol malonaldialdehyde mg^{-1} protein[b]			%OH·-scavenging[c]		
(mM)	BA	SD	FP	BA	SD	FP	BA	SD	FP
0.2	14.6±0.94[1]	4.8±0.58[1]	2.3±0.50[1]	15.7±1.44[1]	16.4±2.20[1]	1.5±1.07[1]	28.7±1.78[1]	15.9±0.60[1]	2.5±1.40[1]
0.4	14.2±0.52[1]	6.6±0.22[1]	10.2±1.16[2]	21.3±1.78[2]	27.5±0.80[2]	7.4±0.62[2]	32.2±2.52[1,2]	18.5±0.68[2]	6.7±1.84[2]
0.6	14.1±1.08[1]	9.6±0.14[2]	11.4±1.62[2,3]	39.3±0.81[3]	38.3±2.45[3]	15.7±0.90[3]	34.9±2.11[2]	22.6±1.22[3]	10.4±1.31[3]
0.8	15.2±0.71[1]	12.8±1.48[3]	12.6±1.76[3]	41.4±1.62[3,4]	42.5±1.84[3]	26.2±1.88[4]	44.2±1.66[3]	34.6±1.54[4]	24.8±2.12[4]
1.0	15.4±1.40[1]	20.8±1.82[4]	18.8±0.91[4]	44.2±2.12[4]	52.2±3.54[4]	49.2±2.67[5]	0	0	0

[a] Values are mean with standard error of measurements (n = 3). Means with same superscript in a given column are the same at $P<0.05$

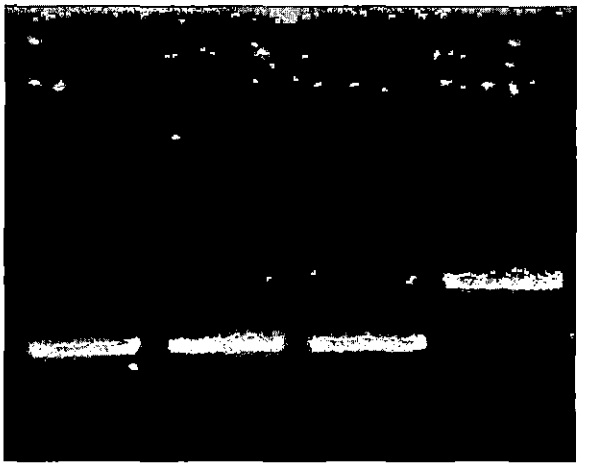


Fig.14. Gel electrophoresis pattern exhibiting the effect of byak-angelicol (BA) on Fenton-mediated plasmid DNA damage. Lane 1, native DNA; Lanes 2-3, native DNA + Fenton reagent + BA (2.5 and 5μg ml^{-1}, respectively); Lane 4, native DNA + Fenton reagent

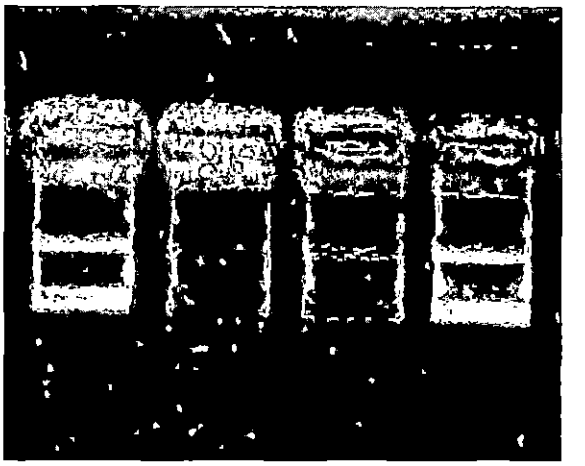


Fig. 15. Gel electrophoresis pattern of plasmid pBR322 exposed to Fenton reagent in the presence and absence of different concentrations of furopinnarin (FP). Lane 1, native DNA; Lane 2, native DNA + Fenton reagent; Lanes 3-5, native DNA + Fenton reagent + FP (2.5 and 5 μg ml^{-1}, respectively).

Table 18. Quantification of DNA bands in different lanes of Fig. 14

Sample	% supercoiled DNA	% protection
native DNA	95.26	100
DNA + Fenton reagent (A)	8.48	0
A + byak-angelicol (2.5 μg ml^{-1})	89.71	93.60
A + byak-angelicol (5 μg ml^{-1})	78.56	80.76

Table 19. Quantification of DNA bands in different lanes of Fig. 15

Sample	% supercoiled DNA	% protection
native DNA	88.73	100
DNA + Fenton reagent (A)	12.03	0
A + furopinnarin (2.5 μg ml^{-1})	42.91	40.20
A + furopinnarin (5 μg ml^{-1})	80.64	89.45

effective against two Gram-negative bacteria at the same concentration range; *E. coli* being more resistant to all of them. In general, BA and FP were more potent than SD (Table 20).

Table 20. Concentration-dependent antibacterial activities of the isolated byak-angelicol (BA), sphondin (SD) and furopinnarin (FP)[a]

Furano-coumarin (μM)	*Listeria monocytogenes*			*Escherichia coli*			*Salmonella sp.*			*Bacillus subtilis*		
	BA	SD	FP	BA	SD	FP	BA	SD	FP	BA	SD	FP
100	56.3±1.4	52±0.7	63.7±2.1	34.3±1.8	38.8±2.8	48.3±1.1	70.5±0.4	51.1±0.9	73.7±0.4	75.6±0.4	68.6±1.6	70.1±0.5
80	42.6±0.8	34±1.5	55.5±1.4	22.4±2.5	26.7±2.5	30.4±2.4	52.6±0.8	47.5±0.6	55.5±0.7	62.1±0.7	53.9±0.5	52.5±0.4
60	20.6±1.6	30±1.8	42.8±1.7	23.7±2.2	13.1±1.6	26.6±0.8	42.5±0.8	35.7±0.5	32.5±0.8	56.3±0.9	44.4±0.8	46.8±0.6
40	14.3±0.6	15±1.1	20.4±2.2	10.1±1.5	6.8±1.2	14.2±0.4	36.2±0.6	29.1±0.4	11.3±0.3	41.3±0.3	26.2±0.6	35.4±0.5
20	0	0	5.7±0.8	0	0	8.1±0.9	23.7±0.3	8.4±0.7	9.6±0.2	25.8±0.2	15.7±0.5	11.2±0.3

[a]Values (% inhibition) are mean with standard error of measurements (n = 3).