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Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants

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Abstract

Total antioxidant capacities of 133 Indian medicinal plant species sampled from 64 families were assessed by ABTS, DPPH and FRAP assays, and their total phenolic contents measured by Folin–Ciocalteu assay. These species exhibited a broad range of antioxidant activities, varying from 0.16 to 500.70 mmol TEAC/100 g DW in the ABTS assay. The antioxidant activity values similarly varied with the DPPH and FRAP assays. Significant and positive linear correlations were found between total antioxidant capacities and phenolic contents ($R = 0.89$–0.97), indicating that phenolics were the dominant antioxidant constituents in the tested medicinal plants. Preliminary identification of the major phenolic compounds from 83 selected medicinal plants by reversed-phase HPLC revealed phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, and quinines. The fruit of *Terminalia chebula*, pericarp of *Punica granatum* and gall of *Rhus succedanea* showed very high levels of hydrolysable tannins, and the gum of *Acacia catechu* presented very high levels of catechin and epicatechin in addition to tannins. Major phenolics in many of the medicinal plants were identified for the first time (e.g., *Euphorbia lathyrus*, *Ipomoea turpethum*, and *Picrorrhiza kurroa*). This systematic investigation of a large number of Indian medicinal plants proved important for understanding their chemical constituents and functionality in Ayurvedic medicine, and contributes to the search for natural sources of potent antioxidants.

Keywords: Antioxidant activity; Ayurveda; Indian medicinal plants; Phenolic antioxidants; Phenolics; ABTS; DPPH; FRAP

1. Introduction

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz et al., 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park & Pezzutto, 2002).

Free radicals, produced as a result of normal biochemical reactions in the body, are implicated in contributing to cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, hair loss, and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Beal, 1995; Maxwell, 1995; Poulson, Preime, & Loft, 1998). The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Apart from these, plant secondary metabolites such as flavonoids and

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terpenoids play an important role in the defence against free radicals (Devasagayam & Sainis, 2002; Govindarajan, Vijayakumar, & Pushpangadan, 2005; Park & Pezzutto, 2002).

Ayurveda is an ancient system of medicine practiced in India since the Vedic period, about 3,500 years ago. The first recorded Ayurvedic medicine book, *Charaka Samhita*, was written in 600 BC (Schuppan, Jia, Brinkhaus, & Hahn, 1999). The Ayurveda system relies strongly on preventive medicine and promotion of positive health. Ayurvedic preparations called *Rasayanas* are used to promote health. The *Rasayanas* are preparations from several plant extracts, which contain strong antioxidants and are used as rejuvenators or nutritional supplements (Govindarajan et al., 2005; Sharma, Hanna, Kauffman, & Newman, 1992; Thyagarajan et al., 2002).

Medicinal plant parts (roots, leaves, branches/stems, barks, flowers, and fruits) are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins (Cai, Luo, Sun, & Corke, 2004; Kähkönen et al., 1999; Larson, 1988). They have multiple biological effects including antioxidant activity (Tapiero, Tew, Ba, & Mathé, 2002). The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen (Rice-Evans, Miller, & Paganga, 1996). Flavonoids, which are partly responsible for the pigmentation of flowers, fruits and leaves, are subdivided into flavanols, flavonols, flavones, flavanones and anthocyanins based on the saturation of the flavan ring and also their hydroxylation. They occur mostly as glycosylated derivatives, sometimes conjugated with sulphate or organic acids (Youdim, Spencer, Schroeter, & Rice-Evans, 2002).

There have been several studies on the antioxidant activities of various herbs/plants with medicinal values (e.g., Dragland, Senoo, Wake, Holte, & Blomhoff, 2003; Kähkönen et al., 1999; Zheng & Wang, 2001). A systematic assay of antioxidant capacities of 112 Chinese medicinal plants associated with anticancer was conducted earlier in our laboratory (Cai et al., 2004). Fifteen Indian medicinal plants commonly used in Ayurveda were recently reviewed in detail with respect to their antioxidant capacities (Govindarajan et al., 2005). Seven important medicinal plants species used in Ayurveda had been reviewed earlier with detailed data on their secondary metabolites and antioxidant properties (Scartezzini & Speroni, 2000). Auddy et al. (2003) used the 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method and lipid peroxidation assay to evaluate the antioxidant potential of three species, *Sida cordifolia*, *Evolvulus alsinoides*, and *Cyanodon dactylon*, which are used in the treatment of neurodegenerative disorders. A similar study was done using four other plants, *Momordica charantia*, *Glycyrrhiza glabra*, *Acacia catechu*, and *Terminalia chebula* (Naik et al., 2003). Jadhav and Bhutani (2002) studied antioxidant properties of methanolic extracts of 12 Indian medicinal plants using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. However, all these previous studies included only a small number of medicinal plants and used only one assay method. A comparative, multi-method screening of antioxidant activity for a large number of Indian medicinal plants in relation to their phenolic compounds is needed to provide a better understanding of their relative importance as natural antioxidants.

Several different methods are available and have been used to assess the total antioxidant capacity of plant extracts, such as the ABTS assay (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993), the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996), and the oxygen radical absorbance capacity (ORAC) assay (Cao, Alessio, & Buettner, 1993). In the present study, we evaluated the total antioxidant capacities of 133 traditional Indian medicinal plant species from 64 families using an improved ABTS method (Cai et al., 2004; Re et al., 1999), an improved FRAP assay, and a modified DPPH assay. We also estimated the total phenolic contents of these plants using the classical Folin–Ciocalteu reagent, and investigated the relationship between the total antioxidant capacities and phenolic contents in the samples tested. Furthermore, we employed reversed-phase high performance liquid chromatography (RP-HPLC) to identify major phenolic compounds in the Indian medicinal plants with high antioxidant activity. These data will be helpful for comparison of the antioxidant activities and phenolic compounds of different medicinal plants and also useful for understanding their chemical constituents and functionality.

2. Experimental

2.1. Sample collection

A total of 137 samples, representing 133 traditional Indian medicinal plant species form 64 families, were collected from traditional medicine stores in Madras, India. These medicinal plants were harvested, dried, and ready for medicinal preparations according to Ayurveda and Siddha traditions. The scientific names of the species and family studied, code number, and parts used are detailed in Table 1. The plant parts used in this study are the same as those generally used in medicinal preparations in traditional Indian medicine, such as leaves, stems/barks, flowers, fruits, seeds, roots/tubers/rhizomes, or even the whole plant.

2.2. Chemicals and reagents

The compounds 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), $FeCl_3 \cdot 3H_2O$, potassium persulphate, sodium acetate, and sodium carbonate were purchased from Sigma/Aldrich (St. Louis, MO). Folin–Ciocalteu reagent, formic acid, glacial acetic acid, and HPLC grade organic solvents were purchased from BDH (Dorset, England). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)

Table 1
133 Indian medicinal plants (137 samples) studied and total antioxidant capacity and phenolic content of their methanolic extracts[a]

Family and species (code number)	Medicinal parts or substance used	TEAC (mmol/100 g DW)[b]		FRAP assay (μmol/g DW[c])	Total phenolic content (g/100 g DW[d])
		ABTS assay	DPPH assay		
Acanthaceae					
Andrographis paniculata (Burm.f.) Wallich ex Nees. (1)	Whole plant	4.76	0.79	1.77	0.72
Blepharis edulis Pers. (2)	Seed	9.80	11.21	1.64	1.75
Hygrophila auriculata (Schum.) Heine (3)	Seed	7.21	6.89	1.81	1.08
Acoraceae					
Acorus calamus L. (4)	Rhizome	2.55	1.11	0.52	0.49
Aizoiaceae					
Mullogo nudicaulis Lam. (5)	Whole plant	1.25	0.96	0.80	0.96
Amaranthaceae					
Achyranthes aspera L. (6)	Whole plant	3.03	0.88	0.90	0.39
Aerva lanata (L.) Juss. (7)	Whole plant	2.43	0.59	0.88	0.35
Anacardiaceae					
Rhus succedanea L. (8)	Galls	224.83	236.49	104.45	12.13
Semecarpus anacardium L.f (9)	Seed	20.19	26.10	3.15	1.64
Mangifera indica L. (10)	Seed	166.89	184.62	25.32	8.67
Apiaceae (Umbelliferae)					
Anethum sowa Roxb. (11)	Seed	8.28	4.45	1.75	1.45
Carum copticum (L.) Benth. & Hook. F (12)	Seed	42.44	8.87	8.86	3.15
Coriandrum sativum L. (13)	Seed	2.22	2.90	0.33	0.41
Cuminum cyminum L. (14)	Seed	6.31	14.02	1.82	0.78
Foeniculum vulgare Mill. (15)	Seed	6.99	2.63	1.79	1.11
Apocynaceae					
Holarrhena antidysenterica Wall. (16)	Fruit	9.49	4.69	1.83	0.94
Rauvolfia serpentina (L.) Benth. ex Kruz. (17)	Stem	6.66	2.58	0.92	0.93
Aristolochiaceae					
Aristolochia bracteata Retz. (18)	Leaf, stem, pod	4.94	2.61	0.89	0.63
Asclepiadaceae					
Calotropis gigantea (L.) R.Br.(19)	Root	11.82	8.29	1.83	1.28
Gymnema sylvestre (Retz.) R.Br. ex Reomer & Schultes (20)	Leaf	6.90	1.25	1.69	1.31
Hemidesmus indicus R.Br. (21)	Root	17.24	15.76	3.46	2.19
Asteraceae (Compositae)					
Anacyclus pyrethrum L. (22)	Root	4.25	4.54	1.41	0.92
Artemisia abrotanum L. (23)	Leaf	2.43	1.68	0.34	0.49
Eclipta alba (L.) Hassk. (24)	Leaf, flower	2.02	1.24	0.54	0.30
Vernonia anthelmintica (L.) Willd. (25)	Seed	9.82	13.34	3.39	1.66
Berberidaceae					
Berberis aristata DC. (26)	Root	3.59	1.65	0.70	0.36
Bignoniaceae					
Oroxylum indicum (L.) Kunze (27)	Root	5.39	3.51	1.81	0.24
Bombacaceae					
Bombax malabaricum DC. (28)	Gum	55.38	80.12	9.06	5.89
Brassicaceae					
Brassica alba (L.) Boiss (29)	Seed	2.67	1.49	0.17	0.30
Brassica nigra (L.) Koch (30)	Seed	2.82	1.45	0.55	0.32
Lepidium sativum L. (31)	Seed	1.60	1.66	0.65	0.15
Matthiola incana (L.) Ait. f. (32)	Seed	3.64	3.27	0.83	0.47
Burseraceae					
Balsamodendron mukul Hook ex. Stocks (33)	Gum	11.08	7.23	1.90	1.56
Boswellia serrata Roxb. (34)	Gum	0.16	0.01	0.18	0.22
Canarium strictum Roxb. (35)	Gum	0.51	0.20	0.17	0.22
Caesalpinaceae (Leguminosae)					
Caesalpinia bonducella (L.) Roxb. (36)	Seed	0.61	0.13	0.18	0.13

Table 1 (*continued*)

Family and species (code number)	Medicinal parts or substance used	TEAC (mmol/100 g DW)[b]		FRAP assay (μmol/g DW[c])	Total phenolic content (g/100 g DW[d])
		ABTS assay	DPPH assay		
Caesalpinia sappan L. (37)	Heartwood	34.65	28.33	7.96	5.46
Cassia auriculata L. (38)	Leaf, flower	118.63	112.25	67.88	9.47
Cassia fistula L. (39)	Pod	9.38	7.69	1.58	1.01
Cassia tora L. (40)	Seed	7.27	6.01	1.63	0.64
Capparaceae					
Cleome viscosa L. (41)	Seed	2.16	0.85	0.36	0.25
Celastraceae					
Celastrus paniculata Willd. (42)	Seed	0.76	0.35	0.19	0.27
Combretaceae					
Terminalia arjuna (DC.) Wight & Arn. (43)	Bark	73.00	85.64	18.10	4.78
Terminalia bellirica Roxb. (44)	Fruit	132.53	161.30	26.42	9.27
Terminalia chebula Retz. (45)	Fruit	500.70	679.69	85.60	35.63
Convolvulaceae					
Evolvulus alsinoides (L.) (46)	Whole plant	2.27	1.85	0.18	0.31
Ipomoea digitata L. (47)	Root	0.40	0.08	0.17	0.06
Ipomoea turpethum R.Br. (48)	Root	8.40	5.54	1.77	0.69
Cucurbitaceae					
Corallocarpus epigaeus (Rottl.) Clarke (49)	Tuber	0.71	0.36	0.18	0.15
Cucumis sativus L. (50)	Seed	0.51	0.00	0.18	0.07
Mukia scabrella (L.) Arn (51)	Whole plant	1.29	0.36	0.18	0.16
Trichosanthes cucumeria L. (52)	Whole plant	1.51	0.33	0.18	0.18
Cyperaceae					
Cyperus rotundus L. (53)	Root	9.84	9.65	1.74	1.49
Elaeocarpaceae					
Elaeocarpus tuberculatus Roxb. (54)	Seed	2.56	1.10	0.54	0.28
Euphorbiaceae					
Euphorbia hirta L. (55)	Whole plant	38.35	50.66	1.69	3.24
Euphorbia lathyrus L. (56)	Leaf and Seed	4.54	6.01	0.91	1.15
Phyllanthus amarus Schum. & Thonn. (57)	Whole plant	55.05	67.60	9.06	5.70
Ricinus communis L. (58)	Seed	0.65	0.22	0.19	0.11
Fabaceae					
Abrus precatorius L. (59)	Seed	75.98	94.84	13.59	3.97
Dolichos biflorus L. (60)	Seed	4.68	2.35	1.37	0.35
Mucuna pruriens (L.) DC. (61)	Seed	90.80	80.08	13.01	6.15
Psoralea corylifolia L. (62)	Seed	21.08	2.35	3.54	2.53
Fagaceae					
Quercus infectoria Oliv. (63)	Seed	11.71	5.39	1.85	1.64
Flacourtiaceae					
Hydnocarpus kurzii (King) Warb. (64)	Seed	0.54	0.22	0.18	0.12
Gentianaceae					
Gentiana kurroo Roy. (65)	Root	21.38	17.40	5.31	3.90
Clusiaceae					
Garcinia mangostana L. (66)	Pericarp	39.19	26.91	8.56	5.10
Mesua ferrea L. (67)	Seed and pericarp	35.22	56.67	8.99	4.18
Hypoxidaceae					
Curculigo orchioides Gaert. (68)	Rhizome	10.60	6.96	1.69	1.32
Illiciaceae					
Illicium verum Hook. fil. (69)	Fruit	16.22	17.63	1.69	2.37
Lamiaceae					
Ocimum basilicum L. (70)	Leaf	25.06	23.45	7.04	2.63
Ocimum sanctum L. (71)	Leaf	7.05	7.18	0.89	0.98

(*continued on next page*)

Table 1 (*continued*)

Family and species (code number)	Medicinal parts or substance used	TEAC (mmol/100 g DW)[b]		FRAP assay (μmol/g DW[c])	Total phenolic content (g/100 g DW[d])
		ABTS assay	DPPH assay		
Lecythidaceae					
Barringtonia racemosa (L.) Blume ex. DC. (72)	Seed	18.39	18.61	2.64	1.68
Liliaceae					
Aloe littoralis Baker. (73)	Leaf	49.13	53.08	8.68	6.20
Asparagus adscendens Roxb. (74)	Root	1.68	0.37	0.33	0.27
Smilax china L. (75)	Root	8.75	9.70	1.70	1.38
Loganiaceae					
Strychnos nux-vomica L. (76)	Seed	0.61	0.38	0.16	0.11
Strychnos potatorum L. (77)	Seed	1.70	0.71	0.38	0.26
Lythraceae					
Lawsonia inermis L. (78)	Seed	16.33	7.16	2.62	3.68
Malvaceae					
Althea officinalis L. (79)	Seed	1.45	0.67	0.18	0.26
Hibiscus rosa-sinensis L. (80)	Flower	21.15	24.66	5.31	3.15
Menispermaceae					
Anamirta cocculus (L.) Wight & Arn. (81)	Fruit	7.01	7.37	1.52	1.18
Tinospora cordifolia (Lour.) Miers. (82)	Root	4.08	1.75	0.72	0.45
Mimosaceae					
Acacia arabica (Lam.) Willd. (83)	Gum	0.75	0.59	0.17	0.06
Acacia catechu Willd. (84)	Gum	428.62	421.18	124.05	41.47
Entada rheedii Sprengel (85)	Seed	59.40	53.80	16.83	5.60
Moringaceae					
Moringa oleifera Lam. (86)	Seed	0.74	0.47	0.17	0.18
Myricaceae					
Myrica nagi Thunb. (87)	Bark	153.76	149.81	26.53	15.02
Myrisinaceae					
Embelia ribes Burm. f. (88)	Fruit	33.31	16.01	8.82	2.36
Myristicaceae					
Myristica fragrans Houtt. (89)	Seed coat (mace)	26.03	9.70	5.37	1.98
Myristica fragrans Houtt. (89)	Seed (nutmeg)	17.92	13.31	5.12	1.30
Myrtaceae					
Syzygium cumini (L.) Skeels (90)	Seed	85.10	99.16	18.37	3.30
Nyctaginaceae					
Boerhaavia diffusa L. (91)	Root	1.18	0.43	0.18	0.19
Pedaliaceae					
Pedalium murex L. (92)	Fruit	3.43	0.91	0.84	0.49
Pinaceae					
Cedrus deodara (Roxb. ex D. Don) G. Don f. (93)	Wood	10.10	23.68	5.22	1.53
Piperaceae					
Piper chaba Hunter (94)	Fruit	6.34	3.77	1.81	0.88
Piper cubeba L. (95)	Fruit	4.19	1.88	1.42	0.86
Piper longum L. (96)	Fruit	4.76	0.94	1.47	0.68
Piper nigrum L. (97)	Fruit (black)	2.81	0.91	0.72	0.65
Piper nigrum L. (97)	Fruit (white)	1.36	0.50	0.18	0.38
Plantaginaceae					
Plantago ovata Forsk. (98)	Seed	0.79	0.49	0.17	0.10
Plumbaginaceae					
Plumbago rosea L. (99)	Root	43.24	37.66	8.69	4.41
Punicaceae					
Punica granatum L. (100)	Seed	3.53	2.90	0.94	0.51
Punica granatum L. (100)	Pericarp	316.29	394.66	90.70	19.22

Table 1 (*continued*)

Family and species (code number)	Medicinal parts or substance used	TEAC (mmol/100 g DW)[b]		FRAP assay (μmol/g DW[c])	Total phenolic content (g/100 g DW[d])
		ABTS assay	DPPH assay		
Ranunculaceae					
Aconitum ferox Wall. ex Ser. (101)	Root	1.77	1.09	0.18	0.59
Aconitum heterophyllum Wall. (102)	Root	3.49	3.19	0.91	0.46
Nigella sativa L. (103)	Seed	1.66	1.08	0.52	0.35
Rubiaceae					
Adina cordifolia (Roxb.) Hook. f. ex Brandis (104)	Root	10.07	9.32	1.46	1.56
Rubia cordifolia L. (105)	Root	10.49	8.23	1.83	1.15
Spermacoce hispida L. (106)	Whole plant	3.91	2.73	0.92	0.77
Rutaceae					
Feronia elephantum Correa (107)	Pericarp	8.91	2.07	1.79	1.70
Murraya exotica L. (108)	Leaf	10.33	8.57	1.80	1.23
Toddalia aculeata Pers. (109)	Bark	12.06	6.53	0.87	2.03
Sapotaceae					
Mimusops elengi L. (110)	Flower	14.48	17.83	1.78	1.57
Scrophulariaceae					
Bacopa moniera (L.) Pennell (111)	Whole plant	1.44	1.41	0.72	0.31
Picrorrhiza kurroa L. (112)	Root	20.69	21.47	8.99	3.14
Solanaceae					
Datura alba Nees. (113)	Seed	3.67	3.38	0.88	0.47
Solanum nigrum L. (114)	Fruit	3.66	2.53	0.89	0.54
Solanum xanthocarpum Schrad. & Wendl. (115)	Fruit	11.58	11.81	2.72	1.98
Withania somniferum (L.) Dunal (116)	Root	1.13	0.66	0.16	0.16
Sterculiaceae					
Helicteres isora L. (117)	Pod	25.24	27.01	13.44	2.61
Styraceae					
Styrax benzoin Dry. (118)	Gum	12.51	5.77	1.86	1.53
Valerianaceae					
Nardostachys jatamansi (Jones) DC. (119)	Root	2.36	1.50	0.90	0.34
Valeriana officinalis L. (120)	Root	8.42	7.88	1.78	1.42
Verbenaceae					
Gmelina arborea Roxb. (121)	Root	14.55	11.69	1.77	0.88
Phyla nodiflora (L.) Greene (122)	Aerial parts	9.07	13.31	1.83	1.53
Premna herbacea Roxb. (123)	Root	11.46	8.27	1.70	1.77
Vitex negundo L. (124)	Leaf	5.90	6.97	1.44	0.99
Violaceae					
Viola serpens Wall. ex Ging. (125)	Leaf	3.48	3.24	0.91	0.82
Zingiberaceae					
Alpinia chinensis Rosc. (126)	Rhizome	47.17	40.98	6.70	5.44
Alpinia galanga (L.) Willd. (127)	Rhizome	2.54	0.84	0.49	0.35
Curcuma longa L. (128)	Rhizome (long)	22.70	6.43	3.76	2.13
Curcuma longa L. (128)	Rhizome (round)	18.88	7.60	3.70	2.16
Curcuma xanthorrhiza Roxb. (129)	Rhizome	15.63	5.74	3.63	1.29
Curcuma zedoaria (Christm.) Rosc. (130)	Rhizome	0.98	0.23	0.17	0.12
Kaempferia galanga L. (131)	Rhizome	4.78	2.00	0.87	0.41
Zingiber officinale Rosc. (132)	Rhizome	10.26	6.71	1.76	0.78
Zygophyllaceae					
Tribulus terrestris L. (133)	Spines	2.25	1.14	0.54	0.39
Overall mean		27.07	28.05	6.56	2.44

[a] All values were means of three measurements. Code numbers in the parenthesis coincide with code numbers of the medicinal plants in Table 3. 133 medicinal plant species include 137 medicinal plant samples. *Myristica fragrans* Houtt. (89), *Piper nigrum* L. (97), *Punica granatum* L. (100) and *Curcuma longa* L. (128) have two tested samples (different parts used or various genotypes), respectively.

[b] TEAC (Trolox equivalent antioxidant capacity) were assayed by the ABTS and DPPH methods. Data expressed as millimoles of Trolox equivalents per 100 g dry weight (DW).

[c] FRAP, ferric reducing antioxidant power. Data expressed as micromoles of Trolox equivalents (TE) per gram dry weight (DW).

[d] Data expressed as grams of gallic acid equivalents (GAE) per 100 g dry weight (DW).

was from Fluka Chemie AG (Buchs, Switzerland). Authentic standards for various phenolic compounds, such as hydroxybenzoic acids, hydroxycinnamic acids, flavones, flavonols, flavanones, flavanols, isoflavones, coumarins, lignans, quinones, curcuminoids, phenolic terpenoids, phenolic volatile oils (e.g., eugenol, carvacrol, thymol), were obtained from Sigma/Aldrich.

2.3. Extract preparation

The medicinal plants collected were ground to a fine powder (710 μm) by a Kenwood Multi-Mill (Kenwood Ltd., UK) and passed through a 24-mesh sieve. The ground samples were dried to constant weights in a desiccant at room temperature (~23 °C) (Cai et al., 2004). For methanolic extraction, 50 mL of 80% methanol was added to 2 g dried plant material in a conical flask, which was kept at room temperature overnight with occasional shaking. The extract was then filtered using a Millipore filter with 0.45 μm nylon membrane under vacuum at 23 °C. The filtrate was stored at 4 °C until use.

2.4. ABTS assay

Antioxidant activity was measured using a Spectronic Genesys 5 spectrophotometer (Milton Roy, New York, NY) using the improved ABTS method (Cai et al., 2004; Re et al., 1999). The ABTS radical cation ($ABTS^{\cdot+}$) solution was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23 °C in the dark for 16 h. The $ABTS^{\cdot+}$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^{\cdot+}$ solution (3.9 mL; absorbance of 0.700 ± 0.005) was added to 0.1 mL of the test sample and mixed thoroughly. The reaction mixture was allowed to stand at 23 °C for 6 min and the absorbance at 734 nm was immediately recorded. The samples were diluted with 80% ethanol so as to give 20–80% reduction of the blank absorbance with 0.1 mL of sample. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 μM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), expressed as mmol Trolox equivalents per 100 g dry weight of plant material.

2.5. DPPH assay

The traditional DPPH assay (Brand-Williams, Cuvelier, & Berset, 1995) was modified for use in this study. The assay procedure was similar to the ABTS method described above. The DPPH radical ($DPPH^{\cdot}$) solution (60 μM) was prepared in 80% ethanol (Cai, Sun, & Corke, 2003). The same samples of medicinal plant extracts diluted with 80% ethanol during the ABTS assay were used in the DPPH assay. The $DPPH^{\cdot}$ solution (3.9 mL; absorbance of 0.68 ± 0.005 at 515 nm) was added to 0.1 mL of the tested extracts. The reaction for scavenging $DPPH^{\cdot}$ radicals was carried out at room temperature in the dark for 120 min, and then the reduction in absorbance was recorded at 515 nm. A calibrated Trolox standard curve was also made. The results were also expressed as TEAC units (mmol Trolox equivalents per 100 g dry weight of sample).

2.6. FRAP assay

Ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996) and Faria et al. (2005) with some modifications. The FRAP assay reagent was prepared by adding 10 vol of 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate and 16 mL glacial acetic acid), 1 vol of 10 mM TPTZ prepared in 40 mM HCl and 1 vol of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and pre-warmed at 37 °C. This reagent (3 mL) was mixed with 0.1 mL diluted test samples similar to those used for the ABTS and DPPH assays. The mixture was shaken and incubated at 37 °C for 8 min and the absorbance was read at 593 nm. A blank with only 0.1 mL methanol was used for calibration. A standard curve was made with Trolox and the results were expressed as μmol Trolox equivalents (TE) per gram dry weight of sample.

2.7. Determination of total phenolic content

The total phenolic content (TPC) of each sample was estimated using the Folin–Ciocalteu colorimetric method according to Liu et al. (2002) and Cai et al. (2004) with minor modifications. Appropriately diluted test sample (0.2 mL) was reacted with 0.5 N Folin–Ciocalteu reagent for 4 min at room temperature. The reaction was then neutralized with saturated sodium carbonate (75 g/L) and allowed to stand for 2 h in the dark at room temperature. Later the absorbance of the resulting blue colour was measured at 760 nm with a spectrophotometer. Quantification was done on the basis of a standard curve with gallic acid. Results were expressed as gram of gallic acid equivalents (GAE) per 100 g dry weight.

2.8. RP-HPLC analysis

RP-HPLC analysis was conducted on a Hewlett–Packard HPLC System (HP 1100 series, Waldbronn, Germany), consisting of a binary pump and a diode-array detector (DAD) and equipped with a 250×4 mm i.d., 5-μm, Nucleosil 100-5 C18 column (Agilent Technologies, Palo Alto, CA). The chromatographic conditions followed a previously reported method (Cai et al., 2004) with minor modifications (solution A: 2.5% formic acid; solution B: 100% methanol; gradient elution program: 0 min, 5% B; 15 min, 30% B; 40 min, 40% B; 60 min, 50% B; 65 min, 55% B; and 90–98 min, 100% B). Flow rate was 0.8

mL/min and injection volume was 20 μL. Detection was monitored at different wavelengths (around λ_{max}) for various phenolic compounds, i.e., 280 nm for hydroxybenzoic acids, tannins, flavanones, flavanols, isoflavones, lignans, quinones, phenolic diterpenes, and some volatile oils (aromatic compounds); 320 nm for hydroxycinnamic acids, flavones, and coumarins; 370 nm for flavonols and chalcones; 420 nm for curcuminoids and anthraquinones, and 520 nm for anthocyanins.

2.9. Statistical analysis

All determinations of antioxidant capacity by ABTS, DPPH, and FRAP assays and measurements of TPC were conducted in triplicate. The reported value for each sample was calculated as the mean of three measurements. Correlation coefficients (R) and coefficients of determination (R^2) were calculated using Microsoft Excel 2000.

3. Results and discussion

3.1. Total antioxidant capacity and phenolic content

The total antioxidant capacities and phenolic contents of 137 methanolic extracts from 133 Indian medicinal plant species of 64 families were systematically assessed. The results of three in vitro assays (ABTS, DPPH, and FRAP) for antioxidant properties of the 137 samples are given in Table 1. The TEAC values of ABTS assay exhibited extremely large variation from 0.16 to 500.70 mmol Trolox equivalents per 100 g dry weight (mmol TEAC/100 g DW). The mean value of all tested medicinal plants was 27.07 mmol TEAC/100 g DW. The total antioxidant capacity determined by the DPPH assay also showed a wide variation in TEAC values from 0.00 to 679.69 mmol per 100 g dry weight (DW) with an average of 28.05 among the 137 medicinal plant samples. Similar to the results of the ABTS assay, high antioxidant capacities were found in the same set of species. The values of FRAP assay were expressed as μmol TEAC per g DW of the sample (μmol TEAC/g DW). The FRAP values of the 137 samples varied from 0.16 to 124.05 μmol TEAC/g DW with a mean value of 6.56. The total phenolic contents (TPC) of these samples were estimated concurrently using the classical Folin–Ciocalteu colorimetric method. It was found that TPC of the 137 samples also showed significant variation, ranging from 0.06 to 41.47 g of gallic acid equivalents (GAE)/100 g dry weight (DW) with a mean of 2.44 g GAE/100 g DW.

The percentage distribution of different classes of TEAC values of the tested plants by the ABTS assay is shown in Fig. 1. Of the 137 samples studied, eight samples (6%) had very high antioxidant capacity (>100.01 mmol/100 g DW). Nearly half of the samples (69 samples, 50%) had TEAC values between 5.01 and 100.00 mmol/100 g DW, and 46 samples (34%) between 1.01 and 5.00 mmol/100 g DW. Only 14 samples (10%) showed very low antioxidant activity (less than 1.00 mmol/100 g DW). The distribution chart indicated that most of the Indian medicinal plant extracts had intermediate levels of total antioxidant capacities, similar to the previous findings in 112 traditional Chinese medicinal (TCM) plants (Cai et al., 2004).

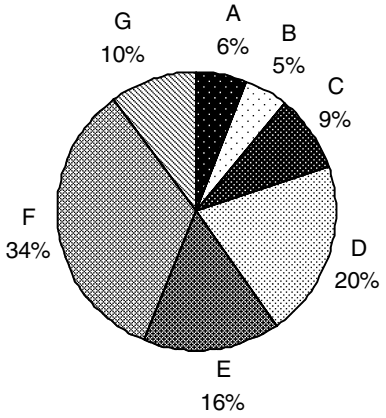

Fig. 1. Distribution (percentage) of 133 Indian medicinal plants (137 samples) among different ranges of total antioxidant capacity assayed by using the ABTS method (TEAC value, mmol/100 g DW). A: >100.01; B: 50.01–100.00; C: 25.01–50.00; D: 10.01–25.00; E: 5.01–10.00; F: 1.01–5.00; G: <1.00.

Although both the present and previous studies (Cai et al., 2004) used the same ABTS assay method, the calculation and unit of TEAC value were slightly different. In order to compare the present results with the results reported in Cai et al. (2004), all the TEAC (μmol/100 g DW) values of the 112 TCM plants by ABTS assay were multiplied by a conversion coefficient (40/1000) and expressed as mmol/100 g DW. It was found that the overall mean TEAC value of the 137 Indian medicinal samples was 27.07 mmol/100 g DW (Table 1), lower than the overall mean ($941.1 \times 40/1000 = 37.64$ mmol TEAC/100 g DW) of the 112 TCM plants (Cai et al., 2004). However, the mean TEAC value of top 20 Indian samples was 137.9 mmol/100 g DW, similar to the mean value of 155.0 mmol/100 g DW of the top 20 TCM plants. The highest antioxidant capacity (500.70 mmol TEAC)/(100 g DW) in this study was found in the fruit of *T. chebula*, while the highest antioxidant capacity (692.9 mmol TEAC)/(100 g DW) was found in the gall of *Rhus chinensis* among the 112 TCM plants (Cai et al., 2004). The differences in TEAC values between the two studies are apparently attributable to different medicinal species/parts that are traditionally used in different cultural practices.

3.2. Relationships among the estimates of total antioxidant capacities with ABTS, DPPH and FRAP assays

To evaluate the suitability and reliability of the three-assay methods used to determine the total antioxidant capacities of the 137 Indian medicinal plant samples, we performed linear regression and correlation analyses of the values of total antioxidant capacity obtained by these

Table 2
Correlations (R and R^2) between different antioxidant capacity parameters (by ABTS, DPPH, and FRAP assays) and total phenolic contents (TPC) of 133 Indian medicinal plants ($n = 137$ samples)

R (R^2)[a]	ABTS	DPPH	FRAP
DPPH	0.9866*** (0.9734)		
FRAP	0.9618*** (0.8535)	0.8810*** (0.7762)	
TPC	0.9690*** (0.9390)	0.9378*** (0.8789)	0.8941*** (0.7995)

[a] R, correlation coefficient. R^2, coefficient of determination. The values in parentheses represent the R^2 values.
*** Significance level at $P < 0.001$.

methods. The correlation coefficients (R) and coefficients of determination (R^2) are given in Table 2. All R-values were positive at the $P < 0.001$ significance level, indicating that the values of antioxidant capacities assayed by the three different methods were highly correlative. These results showed that the three assay methods were all suitable and reliable for assessing total antioxidant capacities of plant extracts, although there were some samples showing differences in total antioxidant capacities between assay methods in the present study.

Fig. 2a and Table 2 show a highly significant linear correlation ($R^2 = 0.9734$ and $R = 0.9866$) between the total antioxidant capacities evaluated by ABTS and DPPH assays of the 137 samples. The R-values between ABTS and FRAP assays and between DPPH and FRAP assays were 0.9618 and 0.8810, respectively. Although these two

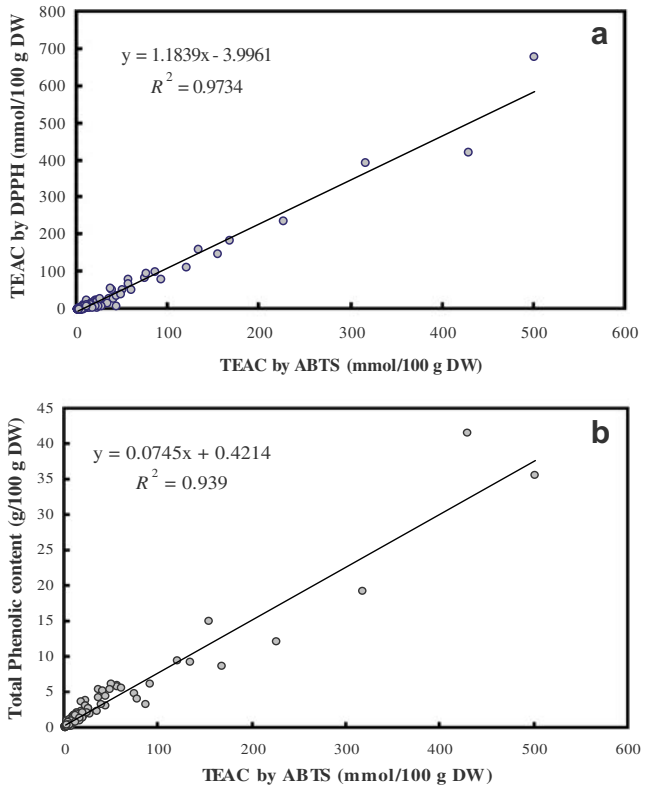

Fig. 2. Relationship between the total antioxidant capacities (TEAC, mmol/100 g DW) by ABTS and DPPH assays and total phenolic content (g gallic acid equivalent/100 g DW) of methanolic extracts from 137 samples (a and b).

R-values are both highly significant ($P < 0.001$), they were lower than the R-value (0.9866) between ABTS and DPPH assays. More samples showed differences in total antioxidant capacities between FRAP and ABTS assays or between FRAP and DPPH assays than between ABTS and DPPH assays. For example, the order of top five samples with the highest antioxidant capacity by FRAP assay was different from those by ABTS or DPPH assays. These results could indicate that ABTS and DPPH assays are more accurate and reliable than the FRAP assay for assessing total antioxidant capacities of plant extracts.

All the three assays of antioxidant capacity used in this study are spectrophotometry-based methods. However, it is not surprising to find the differences in the antioxidant activity measurements among the assays, as each has a different mechanism of action or different reaction conditions. ABTS is a method based on reduction of the 2,2′-azinobis (3-ethylbenzothiazoline sulphonate) radical, and DPPH is a method based on the scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical. Although both $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ have been widely used to measure the antioxidant capacities of natural extracts based on their ability to reduce the radical cation, the reactions of $ABTS^{\cdot+}$ with free-radical scavengers present in the test sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm. The reaction time of the improved ABTS assay is only 6 min, much shorter than that of DPPH assay (120 min in the present study). Moreover, the DPPH assay determines the decrease in sample absorbance at 515 nm, and the coloured compounds such as anthocyanins and carotenoids present in the test sample may have the spectra that overlap with $DPPH^{\cdot}$ at 515 nm and thus interfere with the OD measurements (Arnao, 2000; Prior, Wu, & Schaich, 2005). In contrast, the FRAP assay measures the reducing capability by increased sample absorbance based on the formed ferrous ions, and the assay may not be complete even several hours after the reaction starts, such that a single end-point of the reaction cannot be determined (Prior et al., 2005). Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002) also noted that the FRAP assay has some drawbacks, such as interference, reaction kinetics, and quantitation methods. Considering all these factors, we and several other researchers (e.g., Arnao, 2000; Cai et al., 2004; Lee, Kim, Lee, & Lee, 2003; Shan, Cai, Sun, & Corke, 2005) favour the improved ABTS assay, which was rapid, robust and accurate for systematically assessing total antioxidant capacity of crude

extracts from plant materials on a large scale. However, it has been recommended that at least two methods be used due to the differences between the test systems investigated (Schlesier, Harwat, Bohm, & Bitsch, 2002).

3.3. Relationship between total antioxidant capacity and phenolic content

The strong correlation between the total antioxidant capacity and phenolic content of the 137 Indian medicinal plant samples was firmly established (Table 2, Fig. 2b). A large number of samples with a suitable range of parameter values can provide reasonable R^2 values and representative correlation. The total antioxidant capacity (ABTS assay) and total phenolic content for all 137 samples was positively correlated (Table 2: $R = 0.9690$; Fig. 2b: $R^2 = 0.9390$; $P < 0.001$). Significant correlations were also found between the total antioxidant capacity assayed by DPPH or FRAP method and phenolic content of the 137 samples ($R = 0.9378$ or 0.8941, $P < 0.001$, Table 2), but the correlations were lower than that between the TEAC value by ABTS assay and the total phenolic content.

Previous studies have found that phenolic compounds are major antioxidant constituents in selected herbs, vegetables and fruits, and there are direct relationships between their antioxidant activity and total phenolic content (Dorman, Bachmayer, Kosar, & Hiltunen, 2004; Velioglu, Mazza, Gao, & Oomah, 1998). However, the number of the plant samples tested in previous studies is often very limited. In the present study, we systematically investigated 137 samples representing 133 plant species used in Ayurvedic system of medicine. The highly significant correlations obtained in this study support the hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity of medicinal plants. The $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ scavenging activity and ferric reducing antioxidant power could be credibly predicted on the basis of the Folin–Ciocalteu assay for total phenolic content. These results are consistent with our previous findings for 112 TCM plants (Cai et al., 2004).

3.4. Preliminary identification and analysis of phenolic compounds

In the present study, 83 Indian medicinal plants with higher antioxidant capacity (>4 mmol TEAC/100 g DW by ABTS assay, Table 3) were selected from the 133 tested species for preliminary identification of phenolic compounds by RP-HPLC with a DAD. Phenolic compounds (phenolics) can be defined as a large series of chemical constituents possessing at least one aromatic ring bearing hydroxyl and other subconstituents, including their functional derivatives (Strack, 1997). RP-HPLC analysis is the most used method for identification of plant phenolics. The related HPLC methods for most categories of phenolics in plants have been developed (Andrade, Seabra, Valentão, & Aretias, 1998; Santos-Buelga & Williamson, 2003). In particular, a library of the analytical characteristics of more than 100 phenolic standards established by Sakakibara, Honda, Nakagawa, Ashida, and Kanazawa (2003) and our recent study (Cai et al., 2004) provided important reference data (such as retention times, UV/Vis λ_{max}, and spectra shapes) for rapid identification of major phenolic compounds in the plant extracts by RP-HPLC.

Because of the diversity and complexity of natural phenolic compounds in hundreds of medicinal plant extracts, it is rather difficult to characterize every compound and elucidate its structure. It is not difficult, however, to identify major categories of phenolic compounds and representative phenolics (Cai et al., 2004). In the present study, we conducted preliminary identification of representative natural phenolic compounds from selected Indian medicinal plants by cochromatography with dozens of phenolic standards and by comparison with the literature data (Cai et al., 2004; Sakakibara et al., 2003). The results showed that the tested Indian medicinal plants possessed a wide variety of natural phenolic compounds with various molecular structural features. Major types and representative components of natural phenolics identified in the 83 selected medicinal plants are summarized in Table 3. Their known bioactive constituents associated with phenolic structure are also given in Table 3 based on the literature search.

As shown in Table 3, major types and representative components of phenolic compounds identified in the present study mainly included simple phenolic constituents, e.g., phenolic acids (hydroxycinnamic acids and hydroxybenzoic acids), polyphenolic compounds, e.g., tannins, flavonoids, curcuminoids, coumarins, lignans, and quinones, and some other mixed categories of phenolics, e.g., phenolic terpenoids, phenolic alkaloids, and special phenolic glycosides. The identified phenolic types were similar to the majority of phenolic types found in the 112 TCM plants in a previous study (Cai et al., 2004). Because various phenolic types possess different UV/Vis spectra and molecular polarities, each phenolic type has typical spectral characteristics and relatively fixed retention time range under the reversed-phase chromatographic conditions. The whole HPLC profiles of all identified phenolics were obtained within 90 min. The retention times of various phenolics (including phenolic standards) identified in this study were approximately in the following ranges: 8.0–31.4 min for phenolic acids (except for rosmarinic acid: 46.8 min); 5.7–40.0 min for tannins; 11.0–30.2 min for flavanols (flavan-3-ols); 21.5–51.0 min for glycoside forms of flavones, isoflavones, flavonols, and flavanones, and 24.8–75.0 min for their aglycone forms; 44.0–75.6 min for chalcones; 23.5–41.5 min for anthocyanins; 20.8–48.6 min for coumarins; 79.0–82.4 min for curcuminoids; 47.5–86.6 min for quinones; 46.0–88.7 min for lignans; 65.0–81.5 min for phenolic diterpenes; and 62.0–90.0 min for some volatile oils (e.g., aromatic compounds: 62.4 min for eugenol, 75.1 min for carvacrol). Their maximum

Table 3
Major phenolic compounds of 83 selected Indian medicinal plants with high TEAC value (>4.00 mmol/100 g DW by ABTS assay)

Scientific name (code number[a])	Major type (representative components) of phenolic compounds	
	Bioactive constituents from references[b]	HPLC-DAD identification in this study[c]
Abrus precatorius L. (59)	Flavones, flavonol glycosides, triterpenoid saponins	Very high content of hydrolysable tannins (gallotaninns), flavonoids
Acacia catechu Willd. (84)	Tannins (phlobatanin, protocatechu tannin), catechu-tannin acid, (+)-catechin, flavonoids, polysaccharides	Catechin, epicatechin, hydrolysable tannins
Adina cordifolia (Roxb.) Hook. f. ex Brandis (104)	Flavanoids, monoterpenoid alkaloid (cadambine)	High content of chlorogenic acid (phenolic acid), flavones
Aloe littoralis Baker. (73)	Littoraloin, deacetyllittoraloin, *C*,*O*-diglucosylated oxanthrone (littoraloside), coumarins, naphthalenes and flavonoids	Hydrolysable tannins, phenolic acids, oxanthrones
Alpinia chinensis Rosc. (126)	Essential oils, diterpenoids	Very high levels of phenolic volatile oils, phenolics acids
Anacyclus pyrethrum L. (22)	No information on phenolics	Phenolic terpenoids, phenolic acids (chlorogenic acid), flavone glycosides
Anamirta cocculus (L.) Wight & Arn. (81)	Sesquiterpenes (picrotoxin derivatives), triterpenoids, alkaloids (berberine, magnoflorine)	Phenolic terpenoids, phenolic acids
Andrographis paniculata (Burm.f.) Wallich ex Nees. (1)	Diterpenoids (andrographolide, neoandrographolide, homoandrographolide), flavonoids (flavone glycosides)	Phenolic acids (chlorogenic acid), flavonol glycosides, phenolic terpenoids
Anethum sowa Roxb. (11)	Essential oils (carvone, limonene, dillapiole), biphenyl derivatives	Phenolic volatile oils (carvacrol, estragole), phenolic acids (*p*-hydroxybezoic acid, chlorogenic acid), flavonol glycosides
Aristolochia bracteata Retz. (18)	Carboxylic acid derivatives (aristolochic acid)	Phenolic acids (hydroxycinnamic acids), volatile oils
Balsamodendron mukul Hook ex. Stocks (33)	Triterpenes (myrrhanol A and myrrhanone A)	Many kinds of terpenoids (including phenolic terpenoids)
Barringtonia racemosa (L.) Blume ex. DC. (72)	No information on active compounds from the seeds	Flavonoids, phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid)
Blepharis edulis Pers. (2)	Benzoxazine glucoside, benzoxazolone	Phenolic acids (*p*-hydroxybenzoic acid), flavone glucosides
Bombax malabaricum DC. (28)	Phenolic acids, xanthone (mangiferin), naphthoquinone, sesquiterpene lactone, flavonols	Phenolic acids, but others were not isolated and identified by HPLC under current chromatographic conditions
Caesalpinia sappan L. (37)	Flavonoids (chalcones, brazilin, 4-*O*-methylsappanol protosappanin A, caeasalpin J, homoisoflavones)	High content of tannins, chalcones, flavonols
Calotropis gigantea (L.) R.Br. (19)	Oxypregnane oligoglycosides (calotroposides A and B), Triterpenoids from root bark	Chlorogenic acid and its derivatives
Carum copticum (L.) Benth. & Hook. f (12)	Essential oils (thymol, γ-terpinene, β-pinene, *p*-cymene), phenolic glucosides	Phenolic volatile oils (very high level of thymol), phenolic acids (chlorogenic acid), coumarins
Cassia auriculata L. (38)	Anthraquinone glycosides, terpenoid glycosides, protoanthocyanidin	Flavonol glucosides, hydroxyanthraquinones and their glycosides, phenolic acids (gallic acid)
Cassia fistula L. (39)	Flavonoids (catechin, flavone glycosides), proanthocyanidins, anthraquinones, triterpene derivatives	Hydroxyanthraquinones (rhein, emodin, physcion, chrysophanol), phenolic acids, tannins (proanthocyanidins)
Cassia tora L. (40)	Many kinds of anthraquinones	Hydroxyanthraquinones (aloe-emodin, rhein, emodin, chrysophanol, physcion) and their glucosides
Cedrus deodara (Roxb. ex D. Don) G. Don f. (93)	Neolignans, (−)-matairesinol, (−)-nortrachelogenin, centdarol, himachlol, lawsaritol, allohimachlol, dihydroflavonols	Lignans (neolignans), phenolic volatile oils (sesquiterpenoids), flavonoids
Cuminum cyminum L. (14)	Essential oils (cumin aldehyde, cuminal, β-pinene, γ-terpinene, safranal)	Phenolic volatile oils, phenolic acids (chlorogenic acid), flavanoids, coumarins
Curculigo orchioides Gaert. (68)	Phenolic glucosides (curculigoside A, B and C, orchiosides A and B), triterpene glycosides, 2,6-dimethoxyl benzoic acid	Many known/unknown phenolic glucosides
Curcuma longa L. (128)	Curcuminoids (curcumin, bisdemethoxycurcumin, demethoxycurcumin), essential oils (sesquiterpenoids)	Curcuminoids (curcumin, bisdemethoxycurcumin, demethoxycurcumin)
Curcuma xanthorrhiza Roxb. (129)	Curcuminoids (curcumin, bisdemethoxycurcumin, demethoxycurcumin), essential oils (sesquiterpenoids: xanthorrhizol)	Curcuminoids (curcumin, bisdemethoxycurcumin, demethoxycurcumin), phenolic volatile oils (xanthorrhizol)

Table 3 (*continued*)

Scientific name (code number[a])	Major type (representative components) of phenolic compounds	
	Bioactive constituents from references[b]	HPLC-DAD identification in this study[c]
Cyperus rotundus L. (53)	Sesquiterpene hydrocarbons [(−)-isorotundene, (−)-norrotundene], sesquiterpene alkaloids (rotundines A, B, and C)	Phenolic volatile oils (sesquiterpenoids), flavonoid glycosides, phenolic acids
Dolichos biflorus L. (60)	Tannins	Phenolic acids (hydroxybenzoic acids) and tannins
Embelia ribes Burm. f. (88)	Benzoquinone derivatives (embelin, embelinol, embeliaribyl ester, embeliol)	Very high content of hydroxybenzoquinones
Entada rheedii Sprengel (85)	Phenylacetic acid derivatives, sulfur-containing amides, saponins	Tannins, flavonoids, phenolic acids (hydroxybenzoic acids)
Euphorbia hirta L. (55)	Gallic acid, hydrolysable tannins, flavonoids (quercitrin, myricitrin)	High level of hydrolysable tannins (ellagitannins and gallotannins), phenolic acids (gallic acid), flavonoids (flavonol glycosides)
Euphorbia lathyrus L. (56)	No information on phenolics from references	High levels of flavones and flavonol glucosides
Feronia elephantum Correa (107)	No information on phenolics in the pericarp	Phenolic acids (gallic acid, hydroxybenzoic acid), other compounds were not well separated by HPLC
Foeniculum vulgare Mill. (15)	Essential oils, hydroxycinnamic acid derivatives, flavonoids and their glycosides	Phenolic acids (caffeoylquinic acid derivatives), flavonols and flavones and their glycosides, coumarins, phenolic volatile oils
Garcinia mangostana L. (66)	Xanthones (mangostins, garcinone-E, methoxy-β-mangostin, garcimangosone A, garcimangosone B, garcimangosone C)	High concentrations of xanthones (α-mangostin, β-mangostin, γ-mangostin)
Gentiana kurroo Roy. (65)	Flavone-*C*-glucosides (isovitexin), iridoid glucosides, xanthones	Phenolic acids (coumaric acid, ferulic acid), many kinds of flavone glucosides
Gmelina arborea Roxb. (121)	Iridoid glycosides (gmelinosides) in leaves, keto-lignans (arboreal, arborone, gemelanone) in heartwood	Phenylpropanoid glycosides, lignans
Gymnema sylvestre (Retz.) R.Br. ex Reomer & Schultes (20)	Flavonoid compounds, antisweet saponins (oleanane-toye triterpenes, e.g., gymnemic acids)	Flavonoids (quercetin and kaempferol glycosides), phenolic triterpenoids, phenolic acids
Helicteres isora L. (117)	Flavones (trifolin and hibifolin), flavonoid glucuronides, rosmarinic acid, neolignans (helicterins)	Phenolic acids (rosmarinic acid and its derivatives), flavonoids (flavones and their glycosides)
Hemidesmus indicus R.Br. (21)	2-Hydroxy-4-methoxybenzaldehyde, acyclic triterpenic acid, acyclic diterpenic ester and monocyclic sesterterpene ester, and other triterpenes	Phenolic acids (caffeic acid), 2-hydroxy-4-methoxybenzaldehyde
Hibiscus rosa-sinensis L. (80)	Anthocyanins, cyclopropenoids	Phenolic acids (chlorogenic acid), hydrolysable tannins, flavonols and their glycosides, anthocyanins
Holarrhena antidysenterica Wall. (16)	Phenolic acids (ferulic acid), steroidal alkaloids	Phenolic acids (hydroxybenzoic acid, chlorogenic acid, ferulic acid)
Hygrophila auriculata (Schum.) Heine (3)	Triterpenes	Very high content of ferulic acid (phenolic acid)
Illicium verum Hook. fil. (69)	Essential oils (anethole), phenylpropanoid glucosides, lignans, sesquiterpenoids (veranisatins A, B and C)	Phenolic volatile oils (anethole)
Ipomoea turpethum R.Br. (48)	No information on phenolics from references	Phenolic acids (gallic acid, vanillic acid)
Kaempferia galanga L. (131)	Essential oils, flavonoids, *p*-methoxycinnamic acid, ethyl *p*-methoxycinnamate	Phenolic volatile oils, flavonols (kaempferol), phenolic acids (hydroxybenzoic acids)
Lawsonia inermis L. (78)	*p*-coumaric acid, apiin, apigenin, luteolin, and cosmosiin, triterpenoids (lawsowaseem and lawsoshami)	High levels of flavonol and flavone glucosides, phenolic acids (protocatechuic acid)
Mangifera indica L. (10)	Gallotannins	Gallotannins (mono-, di-, and tri-*O*-galloyl-glucoses) and phenolic acids (gallic acid).
Mimusops elengi L. (110)	Pentacyclic triterpenes, flavonoids, phenolic acids, triterpenoid saponin (mimusin, mimosopin) identified from seeds and barks, but no information on phenolics from flowers	Phenolic acids, flavonoids (flavonols and flavones)
Mucuna pruriens (L.) DC. (61)	Dopamine, tetrahydroisoquinoline alkaloids	A large amount of 6-hydroxydopamine, phenolic acids
Murraya exotica L. (108)	Coumarins (murrayatin, murrangatins), furocoumarins, bicoumarins (murradimerins), essential oils	High levels of coumarins, phenolic acids (chlorogenic acid), flavonoids, phenolic volatile oils

(*continued on next page*)

Table 3 (*continued*)

Scientific name (code number[a])	Major type (representative components) of phenolic compounds	
	Bioactive constituents from references[b]	HPLC-DAD identification in this study[c]
Myrica nagi Thunb. (87)	Diarylheptanoids (myricanol, myricanone, 13-oxomyricanol)	High levels of hydrolysable tannins, diarylheptanoid constituents were isolated by HPLC but not identified because of unavailable standards and no detail literature data
Myristica fragrans Houtt. (89)	Essential oils (sabinene, safrole, terpinen-4-ol, elemicin, myristicin), lignans (myrisfragransin)	Phenolic volatile oils
Ocimum basilicum L. (70)	Rosmarinic acid, lithospermic acid, salvigenin, nevadensin, cirsileol, cirsilineol, eupatorin, apigenin, acacetin, genkwanin, cirsimaritin, ladanein, gardenin B	Phenolic acids (very high content of rosmarinic acid, caffeoyl derivatives), phenolic diterpenes, phenolic volatile oils (carvacrol), flavonoids (catechin)
Ocimum sanctum L. (71)	Phenolic acids (rosmarinic acid, chlorogenic acid, caffeic acid), flavonoids (orientin, vicenin, apigenin, luteolin, apigenin glycosides, luteolin glycosides, vitexin, isovitexin, isoorientin), aesculetin, aesculin, eugenol	Phenolic acids (very high content of rosmarinic acid, caffeoyl derivatives), phenolic diterpenes (carnosic acid), phenolic volatile oils (carvacrol), flavonoids
Oroxylum indicum (L.) Kunze (27)	Flavonoids from seeds (chrysin, baicalein, baicalein-7-*O*-glucoside, baicalein-7-*O*-diglucoside)	Flavonoids, phenolic acids
Phyla nodiflora (L.) Greene (122)	Flavonoids (flavone aglycones, flavone sulphates)	Phenolic acids (high content of *p*-coumaric acid), flavonoids (flavones)
Phyllanthus amarus Schum. & Thonn. (57)	Hydrolysable tannins, gallic acid, flavonoids (quercetin, apigenin, rutin), lignans (phyllanthin, hypophyllanthin, geraniin)	Hydrolysable tannins, flavonoids (rutin, quercitrin), phenolic acids (gallic acid)
Picrorrhiza kurroa L. (112)	No information on phenolics from references	Flavonoids (flavone glycosides, flavanone glycosides), phenolic acids
Piper chaba Hunter (94)	Sesquiterpenoids, caryophyllene oxide, phenolic amides: piperchabamides (A, B, C, and D)	Volatile oils, phenolic amides, a few phenolic acids, high level of unknown/identified flavonoids
Piper cubeba L. (95)	Methylenedioxyphenyl lignans: (−)-clusin, (−)-dihydroclusin, (−)-yatein, (−)-hinokinin, and (−)-dihydrocubebin, essential oils (caryophyllene)	Several lignans (HPLC profile was similar to that of reference), phenolic amides, a few phenolic acids (*p*-hydroxybenzoic acid), volatile oils
Piper longum L. (96)	Phenolic amides (piperine, piperanine, pipernonaline), essential oils (caryophyllene, pentadecane)	Volatile oils, phenolic amides, a few phenolic acids
Plumbago rosea L. (99)	Naphthoquinones (plumbagin, droserone, elliptinone, zeylanone), coumarins	Not isolated and identified by HPLC under current chromatographic conditions
Premna herbacea Roxb. (123)	Diterpenoids (sirutekkone)	Phenolic terpenoids, flavonoids
Psoralea corylifolia L. (62)	Coumarins (bakuchiol, psoralen, isopsoralen, corylifolin, corylin and psoralidin), flavonoids (4′-methoxy flavone), chalcone (bavachalcone)	Furocoumarins (psoralen and isopsoralen), flavonoids (flavones and chalcones), phenolic volatile oils
Punica granatum L. (100)	Hydrolysable tannins and phenolic acids (gallic acid)	High levels of hydrolysable tannins (punicalin, punicalagin), gallagic acid, ellagic acid, and gallic acid
Quercus infectoria Oliv. (63)	Tannins, syringic acid, and ellagic acid identified from galls or gallnuts, but no information on phenolics from seeds	Flavonol and flavone glycosides, phenolic acids, phenolic volatile oils
Rauvolfia serpentina (L.) Benth. ex Kruz. (17)	Flavonoids, alkaloids (ajmaline, ajmalicine, reserpine)	Flavonoids (rutin), phenolic acids (*p*-hydroxybenzoic acid,*p*-coumaric acid)
Rhus succedanea L. (8)	Biflavanoids (amentoflavone, agathisflavone, robustaflavone, hinokiflavone, rhusflavanone) from fruits/seeds, but no information on phenolics from galls	Very high levels of hydrolysable tannins (gallotannins), phenolic acids (gallic acid)
Rubia cordifolia L. (105)	Naphthohydroquinones (rubinaphthin A–D), anthraquinones, flavonoids	Hydroxyanthraquinones and their glycosides (alizarin, purpurin, munjistin, ruberythric acid, alizarin-glucoside)
Semecarpus anacardium L.f (9)	Trihydroxyflavone, biflavonoids (biflavones), bhilawanols, anacardoside	Phenolic volatile oils, but flavonoids were not isolated and identified from crude extracts of seeds
Smilax china L. (75)	Steroidal saponins, β-sitosterol, dihydrokaempferol-5-*O*-β-D-glucoside	Phenolic acids
Solanum xanthocarpum Schrad. & Wendl. (115)	Steroidal alkaloid (solasodine)	Very high content of phenolic acids (chlorogenic acid, caffeic acid)
Styrax benzoin Dry. (118)	Cinnamic and benzoic acids	Phenolic acids, but a very big peak ($R_t = 47.6$ min) was not identified
Syzygium cumini (L.) Skeels (90)	Gallic acid, tannins, and anthocyanins in fruits	Gallic acid, high levels of tannins, flavonoids

Table 3 (*continued*)

Scientific name (code number[a])	Major type (representative components) of phenolic compounds	
	Bioactive constituents from references[b]	HPLC-DAD identification in this study[c]
Terminalia arjuna (DC.) Wight & Arn. (43)	Tannins, triterpene glycosides (terminoside A), oleane derivatives (arjunic acid, arjunolic acid, arjungenin, arjunetin, and arjunglucoside I)	High levels of tannins (ellagitannins), low levels of flavonoids and terpenoids
Terminalia bellirica Roxb. (44)	Hydrolysable tannins,chebulagic acid, ellagic acid, gallic acid	High level of gallic acid, hydrolysable tannins
Terminalia chebula Retz. (45)	Hydrolysable tannins (chebulanin, punicalagin, terchebin, chebulinic acid), tannic acid, ellagic acid, gallic acid	Very high levels of ellagitannins and gallotannins (punicalagin, chebulanin, corilagin, chebulagic acid, di-/tri-galloyl-glucoses), ellagic acid, chebulic acid, gallic acid
Tinospora cordifolia (Lour.) Miers. (82)	Diterpene glucosides (amritosides), furanoditerpene glycoside (cordifolisides), sesquiterpenes	Many terpenoids were isolated by HPLC, but most could not be identified because there were no corresponding standards
Toddalia aculeata Pers. (109)	Coumarins (5-methoxysuberenon, norbraylin, toddalenone, toddalolactone), alkaloids	Coumarins, phenolic acids (chlorogenic acid), flavones
Valeriana officinalis L. (120)	Sequiterpenes, flavonoid glycoside (linarin, hesperidin), valerenic acid, hydroxyvalerenic acid, essential oils	Flavone glucosides, high content of chlorogenic acid
Vernonia anthelmintica (L.) Willd. (25)	Flavonoids (flavones, chalcones)	Phenolic acids (chlorogenic acid, caffeic acid, other caffeoyl derivatives), flavone glucosides
Vitex negundo L. (124)	Flavonoids (vitexicarpin, vitexoside), triterpenoids (oleanolic acid, betullinic acid and ursolic acid), diterpenes (vitedoin B), lignans (vitedoin), phenolic acids, β-sitosterol	Phenolic acids (*p*-hydroxybenzoic acid, protocatechuic acid), terpenoids (oleanolic acid, ursolic acid), flavonoids
Zingiber officinale Rosc. (132)	Gingerols, shogaols, gingediols, paradols, zingerone, dehydrozingerone, diarylheptanoids (gingerinone)	Phenolic volatile oils (gingerol, shogaol)

[a] Code numbers in the parenthesis coincide with code numbers of the medicinal plants in Table 1.
[b] Main bioactive constituents (associated with phenolic structure) of selected Indian medicinal plants were mainly from the references searched through "Web of Science" and also cited from three reviews (Devasagayam & Sainis, 2002; Govindarajan et al., 2005; Scartezzini & Speroni, 2000).
[c] Identification using RP-HPLC with DAD by cochromatography with dozens of phenolic standards and by comparison with the literature data.

UV/Vis absorbance wavelength ranges have previously been described (Santos-Buelga & Williamson, 2003; Sakakibara et al., 2003; Xiao, Yang, & Hong, 2000).

The preliminary HPLC analysis of major phenolic compounds (Table 3) showed that phenolic acids and flavonoids were widely distributed in most of the tested Indian medicinal plants. About 70% and 53% of the identified samples were found to have phenolic acids and flavonoids, respectively. Phenolic terpenoids or volatile oils and tannins also commonly occurred in the tested plants and were detected in about 35% and 16% of the samples, respectively. Coumarins, lignans, quinones, and curcuminoids were observed in only about 3–8% of the tested plants.

Major phenolic compounds were isolated and identified from many Indian medicinal plants (e.g., *Anacyclus pyrethrum*, *Euphorbia lathyrus*, *Ipomoea turpethum*, and *Picrorrhiza kurroa*) using RP-HPLC under the chromatographic conditions employed for the first time. For instance, several phenolic terpenoids, flavone glycoside, and high content of chlorogenic acid were isolated and identified in the root of *Anacyclus pyrethrum*. Seeds of *Abrus precatorius* had a very high level of hydrolysable tannins, roots of *Adina cordifolia* contained high content of chlorogenic acid, seeds of *Hygrophila auriculata* possessed a high level of ferulic acid, and seeds of *Quercus infectoria* were rich in phenolic acids, flavonoid glycosides, and phenolic volatile oils. The phenolics identified in these medicinal plants have not been reported before. Some of the reviews and references reported that certain Indian medicinal plants contained specific phenolics, but these phenolics were not shown under chromatographic conditions used. For example, the gum of *Bombax malabaricum* and roots of *Plumbago rosea* were reported to contain xanthones, naphthoquinones, flavonols, and coumarins (Lin, Yang, & Chou, 2003; Shahat et al., 2003; Reddy et al., 2003), but we did not find these phenolic compounds in the samples examined.

In summary, this study has revealed that a wide range of total antioxidant capacities and phenolic contents exist among the 133 Indian medicinal plants assayed. A highly significant, positive correlation was found between antioxidant capacity and phenolic content, indicating that phenolic compounds are a major contributor to antioxidant activity in the medicinal plants. Some of the medicinal plants with the strongest antioxidant capacity and the highest phenolic content were screened for their phenolic profiles, such as *T. chebula*, *Punica granatum*, *A. catechu*, *Rhus succedanea*, *Myrica nagi*, and *Cassia auriculata*. By comparing with authentic standards and related literature references, RP-HPLC was used in this study to identify

many known phenolic compounds and major phenolic categories from the 83 selected Indian medicinal plants. Major types of the phenolics in the tested plants were identified as phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, and quinones. In addition, new unknown phenolic compounds were found in some of the Indian medicinal plants. However, these medicinal plants also contain other complex phenolic compounds, especially phenolic terpenoids or volatile oils which are not commonly identified by RP-HPLC. Therefore, the unidentified/unknown phenolic constituents in the tested plants warrant further analysis with the aid of other advanced techniques and equipments (e.g., GC, GC–MS, LC–MS, and NMR). GC–MS and LC–MS analysis of some important medicinal plants is underway. Systematic evaluation of a large number of Indian medicinal plants is useful for understanding their functionality and chemical constituents, and also supports the view that they can be potential sources of potent natural antioxidants.

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