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Antioxidant and free radical scavenging activities of some promising wild edible fruits

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Abstract: In order to identify the potential sources of natural polyphenols with promising antioxidant (AOA) and free radical scavenging activities (FRSA) some under utilized edible fruits were studied for total phenolic contents (TPC), AOA and FRSA. The TPC varied from 7.3 (*Ficus hookeri*, fruits) to 119.2 mg/g GAE (*Emblica officinalis*, fruits), fruit pericarp of *Castanopsis tribuloides* (46.8 mg/g), fruits of *Spondias axillaries* (69.4 mg/g) and seeds of *Emblica officinalis* (81.5 mg/g) were found to have good amounts of TPC. The AOA varied from 8.6 (*Citrullus colocynthus*, seeds) to 80.3% (*Emblica officinalis*, fruits). The fruits of *E. officinalis*, *Spondias axillaries* and *Baccaurea sapida* were found to have good amounts of TPC and high AOA; low IC_{50}, low EC_{50}, reasonably good values of antiradical power (ARP) that support their effectiveness towards protection of DNA nicking and indicating strong FRSA. The IC_{50} values for inhibition of lipid per oxidation measured by ammonium thiocyanate assay ranged from 0.50 to 4.30 mg/ml; fruits (0.50 mg/ml) and seeds (0.92 mg/ml) of *Emblica officinalis*, fruits of *Spondias axillaries* (0.66 mg ml) and *Baccaurea sapida* (0.84 mg ml) showed better inhibition of peroxide formation compared to reference standard, quercetin (1.27 mg/ml). The ferrous ion-chelating capacity in terms of IC_{50} values varied from 0.28 (*E. officinalis*, fruits) to 2.83 mg/ml (*Spondias axillaries*, seeds). Further, the ferric ion chelating capacity of fruits of *Baccaurea sapida* (0.47 mg/ml) and *E. officinalis* (0.15 mg/ml) were observed to be better as compared to standard quercetin (0.66 mg/ml). Non enzymatic reactive oxygen species scavenging activity of the fruit extracts of *E. officinalis* (1.56 mg/ml), *B. sapida* (1.09 mg/ml) and *S. axillaries* (1.24 mg/ml) were found to be potent superoxide radical scavengers. Fruits of *E.officinalis*, *B. sapida* and *S. axillaries* showed reasonably good site specific inhibition of hydroxyl radical induced deoxyribose degradation on the other hand the non site specific inhibition exhibited IC_{50} values of 0.45 (*E. officinalis*, fruits) to 4.01 mg/ml (*Cyphomandra betaceae*, seeds). Promising samples were further assayed for their specific phenolic composition through HPLC and MS/MS which showed that fruits of *E. officinalis* were found to be potential source of caffeic acid; fruits of *B. sapida* of ellagic acid, fruits of *S. axillaries* and *B. sapida* of gallic acid.

Keywords: Antioxidant activity, total phenolic contents, free radical scavenging activity, antiradical power, DNA nicking

Introduction

Fruits are important sources of minerals, fiber and vitamins, which provides essential nutrients for the human health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies. The wild under utilized edible fruits can also play an important role as food supplement. Fruits offer protection against free radicals that damage lipids, proteins, and nucleic acids. Polyphenols, carotenoids (pro-vitamin A), vitamins C and E present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases (Velioglu *et al*., 1998; Spiller, 2001; Prakash and Kumar, 2011). The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, singlet and triplet oxygen, or decomposing peroxides (Javanmardia *et al*., 2003; Prakash *et al.,* 2007a; Prakash *et al.*, 2007b; Singh *et al*., 2009; Prakash and Gupta, 2009; Prakash and Kumar, 2011).

Wild edible fruits are known to possess beneficiary nutrients like vitamins, minerals and polyphenols, which provide health benefits in addition to their nutritional value. Several varieties of locally available wild fruits are commonly consumed and are considered an integral part of ethno-culture

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globally. Many of them have been used traditionally as medicines and also made into sauces, jellies, jams or pickles for human consumption (Leung, 1968; Sundriyal and Sundriyal, 2001; Glew *et al*., 2005). The antioxidant properties of cultivated edible fruits and plants are well investigated; however, there is little data available about the functional properties of under utilized wild edible fruits. The main objective of present studies was to screen and evaluate the antioxidant potential of some commonly used wild edible fruits growing in India. Therefore, twelve most prominently utilized wild fruit species growing in Sikkim Himalayan region of India viz. *Elaeagnus latifoila, Diploknema butyracea, Eriolobus indica, Spondias axillaris, Machilus edulis, Baccaurea sapida, Ficus hookeri, Emblica officinalis, Citrullus colocynthus, Elaeocarpus sikkimensis, Cyphomandra betacea* and *Castanopsis tribuloides* were selected for present investigation.

Materials and methods

Fruit materials

Healthy and disease free wild fruit species such as *Elaeagnus latifola* (local name Muslerhi, family Eleagnaceae), *Diplokenema butyracea* (Chiuree, Sapotaceae), *Eriolobus indica* (Mehel, Rosaceae), *Spondias axillaries* (Lapsi, Anacardiaceae), *Machilus edulis* (Pumsi, Lauraceae), *Baccaurea sapida* (Kusum, Euphorbiaceae), *Ficus hookeri* (Nebara, Moraceae), *Castanopsis tribuloides* (Katus, Fagaceae), *Emblica officinalis* (Amla, Euphorbiaceae), *Citrullus colocynthus* (Indrenni, Cucurbitaceae), *Elaeocarpus sikkimensi* (Bhadrase, Elaeocarpaceae) and *Cyphomandra betacea* (Rukh Tamatar, Solanaceae) were collected from their natural habitats in Sikkim Himalayan region of India. Samples were chopped, dried, powdered (40-mesh) and stored in polythene bags at 4°C till analysis.

Chemicals

The linoleic acid and β-carotene were purchased from Acros, USA; DPPH and authentic standards from Sigma –Aldrich, USA; solvents and other reagents of analytical grade were purchased from E. Merk, India.

Total phenolic content and antioxidant activity

The powdered plant material (10 mg) was extracted with 50% MeOH: H2O (1:1, 2 X 10 ml), overnight at room temperature. The combined extractives were centrifuged at 6000 g for 15 min, filtered and maintained to 20 ml each. In 1.0 ml of extract, 1.0 ml of Folin's reagent (1N) and 2.0 ml of Na_2CO_3 (20%) were added subsequently and mixed properly. It was left at room temperature for 30 min and maintained to 25 ml with water. The absorbance of test mixture was measured at λ_{Max} 725 nm on Varian Cary 50 Spectrophotometer. The Total phenolic content (TPC) in different extracts were measured by the method of Ragazzi and Veronese (1973) and expressed as gallic acid equivalent (GAE) mg/g on dry weight basis. The Antioxidant activity (AOA) in plant extracts was assayed by auto-oxidation of β-carotene and linoleic acid (Emmons and Peterson, 1999) and expressed as per cent inhibition relative to control.

Free radical scavenging activity and reducing capacity

Free radical scavenging activity of the extracts (1.0 mg/ml methanol) was assayed by using 1, 1-diphenyl-2-picryl- hydrazil (DPPH) radical (6 x 10^{-5} M in MeOH) according to Yen and Duh (1994). The inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti radical power (ARP) was estimated as described by Kroyer (2004). Reducing power of extracts (1.0 mg/ml in MeOH) was determined (Apati *et al.*, 2003) by ferric reducing - antioxidant power assay and quercetin was used as reference standard. Reducing power was expressed as ascorbic acid equivalent (1mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power. Inhibition of lipid peroxidation was determined using ammonium thiocyanate (Lee *et al.*, 2002). Ferrous ion and ferric ion chelating capacity was estimated as described by Decker and Welch (1990) and Wong and Kitts (2001) respectively.

DNA nicking assay

DNA nicking assay was performed using super-coiled pBR322 plasmid DNA (Lee *et al*., 2002). Extracts of different concentrations (5 to 20 µg/ml) and DNA (0.5 µg) were incubated for 10 min at room temperature followed by the addition of 10µl Fenton's reagent (30 µM H_2O_2, 50 µM ascorbic acid, 80 µM $FeCl_3$). The reaction mixture was incubated for 30 min at 37°C and analyzed on 1% agarose gel.

Qualitative analysis by HPLC and LC-MS/MS

For HPLC analysis, 1 g of dried and powdered plant material was extracted with MeOH : H_2O (1:1, 1 x 20 ml) for 2 hour at room temperature followed by hydrolysis with 1 N HCl by refluxing on a water bath. The hydrolysate was filtered and fractionated with ethyl acetate (EtOAc, 2 x 10 ml). The solvent from EtOAc soluble fraction was removed under reduced pressure and residue thus obtained was dissolved in MeOH and subjected to HPLC and LC-

MS/MS for the qualitative and quantitative analysis of phenolic contents. The HPLC system Shimadzu LC-10A (Kyoto, Japan) was equipped with dual pump binary system, UV detector and Phenomenex Luna RP, C18 column (4.6 x 250 mm). An API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) was used for LC-MS/MS. Analysis were performed on a turbo ions spray source in negative mode by using focusing potential –400V, entrance potential -10, declustering potential (DP) 25 -60 and collision energy (CE) 15-35. Full scan acquisition was performed scanning from m/z 150 to 700 u at a cycle time of 2 s. MS/MS product ions were produced by collision-associated dissociation (CAD) of the selected precursor ions in collision cell. In all the experiments, quadrupole (Q1) was operated at unit resolution (Prakash *et al*., 2007a; Prakash *et al.*, 2007b).

Statistical analysis

Results are the mean values of three replicates of the same sample and statistical analysis was performed by analysis of variance (ANOVA).

Results and Discussion

Polyphenols form a large group of phytochemicals with excellent antioxidant properties and play an important role as free radical scavengers required in the maintenance of "redox homeostasis" responsible for various degenerative diseases. The total phenolic contents (TPC) showed wide variation (Table 1) from 7.3 (*Ficus hookeri*, fruits) to 119.2 mg/g GAE (*Emblica officinalis*, fruits). Fruit pericarp of *Castanopsis tribuloides* (46.8 mg/g), fruits of *Spondias axillaries* (69.4 mg/g) and *Baccaurea sapida* (51.4 mg/g), fruits (119.2 mg/g) and seeds (81.5 mg/g GAE) of *Emblica officinalis* were found to have good amounts of TPC. In general, fruits were found with high amount of TPC, whereas their under-utilized parts were with moderate levels. The AOA (Table 1) also showed a wide variation ranging from 8.6% (*Citrullus colocynthus*, seeds) to 80.3% (*Emblica officinalis*, fruits). The fruit pericarp (51.6%) of *Castanopsis tribuloides,* fruits (80.3%) and seeds (62.4%) of *Emblica officinalis*, and fruits of *Spondias axillaries* (73.9%), *Baccaurea sapida* (64.7%) and *Cyphomandra betaceae* (50.3%) were found with reasonably good AOA. The AOA of 98% and 92% had been reported in the rhizome of *Alpinia galanga* and leaves of *Ocimum sanctum* respectively (Juntachote and Berghofer, 2005). Fruits of *Aegle marmelos* (75.2%), fruit pericarp of *Castanopsis elegans* (52.4%), seeds of *Litchi chinensis* (50.1%),

Table 1. Antioxidant activity (AOA %) and total phenolic contents (TPC) mg/g plant material expressed as gallic acid equivalent (GAE) of some fruits and their underutilized parts (Dry weight basis)

Plants	Part	AOA	TPC
Castanopsis tribuloides	Fruits	32.5±2.4	8.8±1.5
	Pericarp	51.6 ±4.0	46.8±3.5
Cyphomandra betaceae	Fruits	50.3±3.3	15.4±2.3
	Seeds	38.5±3.1	16.3±1.7
Ficus hookeri	Fruits	21.2±2.5	7.3±1.2
	Pericarp	29.4±2.7	21.2±1.5
Elaeocarpus sikkimese	Fruits	31.8±2.2	18.2±1.4
Spondias axillaries	Fruits	73.9±4.2	69.4±3.6
	Pericarp	26.1±2.1	20.8±2.4
Emblica officinalis	Fruits	80.3±4.3	119.2±2.1
	Seeds	62.4±3.5	81.5±1.6
Diploknema butyraceae	Fruit pulp	19.6±2.1	37.1±4.1
	Pericarp	16.4±2.6	40.4±1.7
Machulis edulis	Fruits	38.1±2.8	12.7±2.5
Elaeagnus latifolia	Fruits	32.1±1.7	24.2±1.9
Citrullus colocynthus	Fruits	50.1±2.4	38.3±2.6
Eriolobus indica	Seeds	8.6±1.4	10.4±1.9
Baccaurea sapida	Fruits	20.5±3.1	23.7±2.2
	Fruits	64.7±2.7	51.4±3.4
CD at P < 0.01	-	2.49	2.21

fruit pericarp of *Malus sylvestris* (51.7%) and *Mangifera indica* (54.8%) have also been reported with good AOA (Prakash *et al.*, 2011). It was observed that plants with good amounts of phenols showed higher AOA, on the other hand, fruits (15.4 mg/g GAE) of *Cyphomandra betaceae* were with low TPC and comparatively better AOA (50.3%). The better AOA of samples with low phenolic content may be due to the presence of individual phenolic units with special high antioxidant activity or some other phytoconstituents (Vinson, 1998). Also, in case of *Diploknema butyracea* fruits the TPC was reasonably in good amounts but with low AOA. The presence of antioxidant enzymes like superoxide dismutase (SOD) and catalase and non enzymatic antioxidants such as anthocyanins, tocopherols, carotenoids, phosphate and vitamin C may also contribute to the

overall observed anti-oxidative effect (Bartsch and Frank, 1996). The total phenols ranging from 2.12 to 69.4g/100g and AOA 42.5% to 98.0% in different parts of *Cassia fistula, Cinnamomum zeylanicum, Moringa oleifera* and *Vitis vinifera* had been reported (Siddhuraju *et al*., 2002; Siddhuraju and Becker, 2003; Jayaprakasha *et al*., 2003).

Plants with promising AOA were further investigated for FRSA using DPPH free radical assay (Table 2) in terms of inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}), anti radical power (ARP) and reducing power (RP). The fruits and seeds of *Emblica officinalis* and fruits of *Baccaurea sapida*, *Eleocarpus sikkimese, Spondias axillaries*, *Cyphomandra betaceae* showed low IC_{50} ranging from 0.016 to 0.044 mg/mg, low EC_{50} from 0.70 to 1.91 mg/mg DPPH, reasonably high values (52.36 to 142.17) of ARP. They also showed high reducing power as evident by their low 0.47 to 1.52 ASE/ml values. The fruits of *Baccaurea sapida* exhibited reducing power in close proximity to standard, quercetin. The stable free radical DPPH has been widely used to test the free radical scavenging ability of various dietary antioxidants (Brand-Williams *et al.*, 1995). Further, it was noticed that *Elaeagnus latifolia* fruits exhibited low ARP (38.44) but good reducing power (0.81 ASE/ml). The EC_{50} 0.4 and 0.30 mg/ml, reducing power 2.6 and 0.9 ASE/ml had been reported in the rhizomes of *Alpinia galanga* and leaves of *Ocimum sanctum* respectively (Juntachote and Berghofer, 2005). The EC_{50} values 0.03 and 0.11 mg/ml in bark and leaves of *Azadirachta indica* had been reported (Sithisarn and Gritsanapan, 2005). The amounts of TPC, AOA and FRSA are largely dependant on the method of analysis, biodiversity, genetic, seasonal and geographical variations (Kim *et al.*, 2006).

Table 2. Free radical scavenging activity (FRSA) measured by using 1,1-diphenyl-2-picryl- hydrazyl (DPPH) in terms of IC_{50} = inhibitory concentration (mg/mg of dry extract); EC_{50} = efficiency concentration (mg/mg DPPH); ARP = anti radical power and reducing power expressed as ascorbic acid equivalent (ASE/ml) of some promising wild fruits and their underutilized parts

Plant	Parts	IC_{50}	EC_{50}	ARP	ASE/m
Emblica officianilis	Fruits	0.016	0.70	142.17	0.63
	Seeds	0.038	1.65	60.65	1.32
Citrullus colocynthus	Fruits	0.120	5.21	19.19	2.33
	Seeds	0.184	8.0	12.50	4.10
Machulis edulis	Fruits	0.102	4.34	23.51	4.63
Elaeagnus latifolia	Fruits	0.060	2.61	38.44	0.81
Elaeocarpus sikkimese	Fruits	0.044	1.91	52.36	1.52
Spondias axillaries	Fruits	0.032	1.39	71.94	1.17
	Seeds	0.075	3.26	30.67	3.55
Castanopsis tribuloides	Fruits	0.127	5.52	18.11	2.14
	Pericarp	0.190	8.26	12.10	2.28
Diploknema butyraceae	Fruit pulp	0.055	2.30	41.83	1.92
	Pericarp	0.104	4.52	22.17	3.21
Cyphomandra betaceae	Fruits	0.039	1.69	59.28	1.68
	Seeds	0.151	6.56	15.24	1.70
Ficus hookeri	Fruits	0.23	10.0	10.08	3.25
	Pericarp	0.109	4.73	21.14	2.76
Eriolobus indica	Fruits	0.081	3.52	28.37	2.51
Baccaurea sapida	Fruits	0.027	1.12	85.40	0.47
Quercetin	Standard	0.021	0.87	115.01	0.51
CD at P < 0.01	-	0.06	0.36	0.24	0.29

Table 3. Free radical scavenging activity assayed by different methods expressed as IC_{50} (mg/ml) of some promising fruits and their underutilized parts (agri-wastes) on dry weight basis. A = Lipid per oxidation, assayed by ammonium thiocyanate method; B = Ferrous ion chelating capacity; C = Ferric ion chelating capacity; D = Inhibition of NBT reduction caused by superoxide anions; E = Site specific inhibition of hydroxyl radical-mediated deoxyribose degradation; F = Non-site-specific inhibition of hydroxyl radical-mediated deoxyribose degradation

Plant	Parts	A	B	C	D	E	F
Elaeocarpus sikkimese	Fruits	1.40	0.94	1.27	4.93	2.64	2.97
Baccaurea sapida	Fruits	0.84	0.60	0.47	1.09	0.58	1.16
Spondias axillaries	Fruits	0.66	1.25	0.96	1.24	2.01	2.53
	Seeds	4.30	2.83	3.75	2.96	2.40	1.92
Cyphomandra betaceae	Fruits	2.24	2.40	2.78	2.87	3.76	1.90
	Seeds	3.13	2.42	2.17	3.63	3.14	4.01
Emblica officinialis	Fruits	0.50	0.28	0.15	1.56	0.32	0.45
	Seeds	0.92	0.86	0.70	2.16	1.92	1.64
Quercetin	Standard	1.27	0.52	0.66	1.85	0.58	1.06
LSD at P < 0.01	--	2.16	1.53	1.79	2.86	2.01	2.52

Promising fruit samples were further subjected to concentration-dependent FRSA using different methods and expressed in terms of IC_{50} values (Table 3). The IC_{50} values for inhibition of lipid per oxidation (LPO) measured by ammonium thiocyanate assay (Table 3) ranged from 0.50 to 4.30 mg/ml; fruits (0.50 mg/ml) and seeds (0.92 mg/ml) of *Emblica officinalis* fruits of *Spondias axillaries* (0.66 mg ml) and *Baccaurea sapida* (0.84 mg/ml) showed better inhibition of peroxide formation compared to reference standard quercetin (1.27 mg/ml). The difference in inhibition mentioned assays could be due to different steps of lipid oxidation, polarity of polyphenols present in the extract and the antioxidative mechanisms exhibited by them (Romero *et al*., 2004). The leaf, fruit and seed extracts of *Sygzium cumini* showed anti-LPO activity that

varied from 49.55% to 94.37%, 25.67% to 74.33% and 9.48% to 52.72%, respectively at 200-1000 mg/ml (Banerjee *et al*., 2005). The ferrous ion-chelating capacity (Table 3) in terms of IC_{50} values varied from 0.28 (*Emblica officinalis*, fruits) to 2.83 mg/ml (*Spondias axillaries*, seeds). Further, the ferric ion chelating capacity of fruits of *Baccaurea sapida* (0.47 mg/ml) and *Emblica officinalis* (0.15 mg/ml) was observed to be better compared to standard quercetin (0.66 mg/ml). Transition metal ions are known to catalyze the formation of free radicals. On the other hand, phenolic compounds can inhibit their formation by chelating with metal ions (Juntachote and Berghofer, 2005; Gulcin, *et al.*, 2007).

Superoxide radical scavenging activity (SRSA) was assessed based on the capacity of the samples selected to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in the presence of riboflavin-light NBT system (non enzymatic O_2 scavenging activity). The results of non enzymatic SRSA of the extracts showed that fruit of *Emblica officinalis* (1.56 mg/ml), *Baccaurea sapida* (1.09 mg/ml) and *Spondias axillaries* (1.24 mg/ml) were found to be potent superoxide radical scavengers (Table 3). The superoxide scavenging capacity studied in six fruits showed more than 75% scavenging activity at 60 mg and 80 mg sample equivalent/µL concentrations of in red ivory and velvet sweet-berry fruit extracts. The pulps of sour plum had higher activities than the peels (Ndhala *et al.*, 2006). Antioxidants are able to inhibit the blue NBT formation (Cos *et al*., 1998; Parejo *et al*., 2002) and thus it is evident from the results that the extracts in study possess anti-oxidative properties.

The effect of extracts was evaluated on hydroxyl radical generated by Fe^{3+} ions and measured by determining the degree of deoxyribose degradation an indicator of thiobarbituric acid malonaldehyde (TBA-MDA) adduct formation in both site specific and non specific deoxyribose assay (Lee *et al*., 2002). Fruits of *Emblica officinalis* and *Baccaurea sapida* showed better site specific inhibition of hydroxyl radical induced deoxyribose degradation as compared to other extracts. The better site specific inhibition activities of these extracts were also found to be efficient Fe^{3+} ion chelators (Table 3). The concentration dependant inhibition of hydroxyl radical observed in non site specific assay exhibited IC_{50} values of 0.45 (*Emblica officianilis*, fruits) to 4.01 mg/ml (*Cyphomandra betaceae*, seeds) The efficient inhibition of hydroxyl radical by these extracts can be supported by their similarity as potent scavengers of DPPH stable radicals. Further, relatively higher site specific inhibition of hydroxyl radical-induced deoxyribose degradation was observed in the ethanolic stem extracts of *Opuntia ficus- indica* than in non site specific assay with the same concentration (Lee *et al*., 2002). The hydroxyl free radical scavenging activity in aged regional wines of Greece determined using deoxyribose method ranged from 38.5% to 59.9% (Arnous *et al*., 2002). The leaf extracts of *Moringa oleifera* was found to be potent hydroxyl radical scavenger with inhibition percentage (35.99% to 88.49%) for non-site-specific and (20.96% to 68.41%) for site-specific in a concentration range of 200-1000 µg/ml (Chumark *et al.*, 2008).

The free radical scavenging effects of promising fruit extracts on Fe^{3+} dependent hydroxyl radicals induced DNA nicking was studied on pBR322 DNA that showed significant reduction in the formation of nicked DNA and increase in native DNA (super coiled). Fruit extracts (20µg/ml) of *Baccaurea sapida*, *Emblica officinalis* and *Spondias axillaries* effectively prevented DNA nicking (Figure 1) and mitigated the oxidative stresses on susceptible biomolecules. The protection offered by the fruit extracts of *Emblica officinalis* fruits (lane 4) and *Baccaurea sapida*, (lane 5) were significantly close to that of 2 U of catalase (lane 3). Present studies together with the previous works suggest the triple synergistic action of polyphenols in scavenging ROS, repairing DNA and metal chelation (Lee *et al*., 2002; Zhao *et al*., 2005).

Promising fruits were assayed for their specific phenolic composition through HPLC (Table 4) and LC-MS/MS (Table 5). The amount of caffeic acid varied from 30.7 to 2231.0µg/g, chlorogenic acid 35.9 to 540.0 µg/g, ellagic acid 72.4 to 899.2, ferulic acid 14.9 to 463.2 µg/g, gallic acid 65.1 to 1879.6µg/g and quercetin 83.5 to 374.2 µg/g. The presence of kaempferol was observed in seeds of

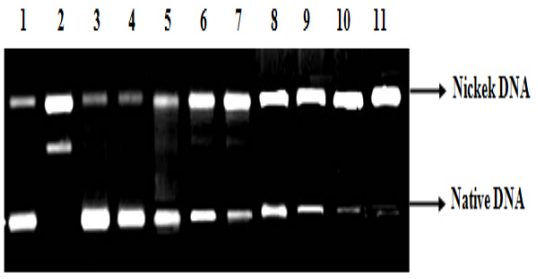

Figure 1. Inhibitory effects of promising fruit extracts on pBR322 DNA nicking caused by hydroxyl radicals.
Lane 1, pBR 322 DNA; Lane 2, DNA + Fenton's reagent; Lane 3, DNA + Fenton's reagent + Catalase (2 units); Lane 4 to 11, DNA + Fenton's reagent + (20 µg/ml) extracts of *Emblica officinalis* fruits, *Baccaurea sapida* fruits, *Elaeocarpus sikkimese* fruits, *Cyphomandra betacea* fruits, *Spondias axillaries* fruits, *Emblica officinalis* seeds, *Spondias axillaries* seeds, *Cyphomandra betacea* seeds respectively.

Table 4. Specific phenolic composition (µg/g dry weight) of some selected fruits and seeds estimated through HPLC. CA = Caffeic acid, CHA = Chlorogenic acid, EA= Ellagic acid, FA=Ferulic acid, GA= Gallic acid, PCA= Protocatechuic acid, KMP = Kaempferol, QC = Quercetin, RT=Rutin

Plant	Parts	A	B	C	D	E	F
Elaeocarpus sikkimese	Fruits	1.40	0.94	1.27	4.93	2.64	2.97
Baccaurea sapida	Fruits	0.84	0.60	0.47	1.09	0.58	1.16
Spondias axillaries	Fruits	0.66	1.25	0.96	1.24	2.01	2.53
	Seeds	4.30	2.83	3.75	2.96	2.40	1.92
Cyphomandra betaceae	Fruits	2.24	2.40	2.78	2.87	3.76	1.90
	Seeds	3.13	2.42	2.17	3.63	3.14	4.01
Emblica officinialis	Fruits	0.50	0.28	0.15	1.56	0.32	0.45
	Seeds	0.92	0.86	0.70	2.16	1.92	1.64
Quercetin	Standard	1.27	0.52	0.66	1.85	0.58	1.06
LSD at P < 0.01	--	2.16	1.53	1.79	2.86	2.01	2.52

Table 5. Phenolic composition identified by MS/MS. A= *Spondias axillaries*, Fruits; B= *Spondias axillaries*, Seeds; C = *Emblica officinalis*, Fruits; D = *Emblica officinalis*, Seeds; E = *Cyphomandra betaceae*, Fruits; F = *Cyphomandra betaceae*, Seeds; G = *Baccaurea sapida*, Fruits; H= *Elaeocarpus sikkimese*, Fruits

Phenols	Plants	Ion full scan MS		MS/MS approach
		$[M-H]^-$	Fragments	Product ion scan
Caffiec acid	A, C, E, F, G, H	179	135	179
Chlorogenic acid	C, F, G	353	191	353
Ellagic acid	A, D, E	301	257, 229	301
Ferulic acid	A, B, C, D, E, F, H	193	178, 149	193
Gallic acid	A, B, C, D , E, F, G, H	169	125	169
Kaempferol	B, F, H	285	133, 151	285
Protocatechuic acid	A, E	153	109	153
Quercetin	A, C, E, G, H	301	151	301
Rutin	C, D	609	301	609

Spondias axillaries, Cyphomandra betaceae and fruits of *Elaeocarpus sikkimese* only whereas rutin was present in low quantities in fruits and seeds of *Emblica officinalis*. Chlorogenic acid was found to be present in fruits of *E. officinalis* and *Baccaurea sapida* and in low amounts in seeds of *Cyphomandra betaceae*. The fruits of *E. officinalis* were found to be potential source of caffeic acid, fruits of *Baccaurea sapida* of ellagic acid, fruits of *Spondias axillaries* and *Baccaurea sapida* of gallic acid. The presence of quercetin was observed in all fruits analysed. The identification of specific polyphenols was further substantiated by MS/MS analysis (Table 5).The fragmentation patterns were in close proximity to earlier report (Sanchez-Rabaneda *et al.*, 2003; Prakash *et al*., 2007a; Prakash *et al.*, 2007b).

The appreciable concentrations of flavonoids, phenolic acids and some other antioxidant phytochemicals present in different fruits and vegetables might be responsible for their efficient free radical-scavenging activity. The dissimilarity in the phyto-constituents and thus in biological activity, between the wild and cultivated plants correlate with the different ecological conditions in which they grow (Conforti *et al*., 2006). The different antioxidants help to scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Yan *et al*., 2006).

Conclusions

Present investigation suggests that the fruits of *Emblica officinalis, Spondias axillaries* and *Baccaurea sapida*, were found to have good amounts of phenols and high AOA; low IC_{50}, low EC_{50}, reasonably good values of ARP which explains their effectiveness towards protection of DNA nicking indicating the strong free radical scavenging activity. The antioxidant capacity of extracts varied according to the system-generating reactive species. It is well known that the performance of a complex mixture such as a plant extract in different antioxidant systems is related to the type of radical generated and to the polarity of the substrate system. The fruits of *E. officinalis* were found to be potential source of caffeic acid; fruits of *B. sapida* of ellagic acid, fruits of *S. axillaries* and *B. sapida* of gallic acid. The application of various methods used in present studies like lipid per oxidation, DPPH radical scavenging, reducing power, metal chelating capacity and DNA nicking to evaluate AOA at multiple concentrations followed by specific phenolic composition might be a justified approach. Further, it holds promise to identify the potential sources of natural polyphenols with promising AOA, FRSA and wide range of other biological activities.

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