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Antioxidant Potential of Some Under-Utilized Fruits

Dhan Prakash [*a], Garima Upadhyay[b], P Pushpangadan[a]

[a]*Amity Institute of Herbal Research & Studies, Amity University, UP, Sector-125, Noida-201303, G B Nagar, UP, India*

[b] *Department of Pomology, College of Horticulture & Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

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Address for Correspondance: dprakash_in@yahoo.com

Abstract— To identify their potential sources extracts of some fruits and their different parts were studied for total phenolic contents (TPC), antioxidant (AOA) and free radical scavenging activities (FRSA). Phenols have profound importance due to their biological and free radical scavenging activities. The amount of TPC varied from 10.5 to 343.2 mg/g and AOA from 20.3 to 96.7%. Fruits of *Caesalpinia mexicana*, *Acacia auriculiformis*, fruit pericarp fibres of *Cocus nucifera*, and fruits of *Emblica officinalis* were found to have high TPC (73.1-343.2 mg/g) and high AOA (68.5-96.7%). Promising fruits were studied for their FRSA and reducing power (RP) measured by DPPH assay where the fruits of *Caesalpinia mexicana*, fruit pericarp fibres of *Cocus nucifera*, fruits of *Emblica officinalis* showed very low IC_{50} ranging from 0.009 to 0.016 mg/ml, EC_{50} from 0.39 to 0.70 mg/mg DPPH and reasonably high values (142.1- 256.3) of anti radical power (ARP), indicating their strong FRSA and reducing power (RP) as evident by their low ASE/ml values (0.42- 1.08). They also showed better inhibition of lipid peroxidation measured by using ferric thiocyanate assay and by using egg yolk compared to reference standard, quercetin. The ferrous and ferric ion chelating capacity of the promising fruits and their underutilized parts in terms of IC_{50} varied from 0.12 (*Emblica officinalis*, fruits) to 2.44 mg/ml (*Mangifera indica*, Seed kernel) and 0.22 (*Caesalpinia mexicana*, fruits) to 2.59 mg/ml (*Litchi chinensis*, fruit peel) respectively. Fruit of *Acacia auriculiformis*, *Caesalpinia mexicana*, *Emblica officinalis,* fruit pericarp fibres of *Cocus nucifera*, were also assayed for their specific phenolic composition through HPLC where the amount of caffeic acid varied from 48.5 to 2231 µg/g, chlorogenic acid 63.8 to 912.1 µg/g, ellagic acid 46.4 to 1429.1 µg/g, ferulic acid 36.7 to 762.9 µg/g, gallic acid 181.6 to 2831.6 µg/g, protocatechuic acid 41.7 to 322.8 µg/g, and quercetin 44.6 to 367.6 µg/g.

Keywords : Total Phenols, Antioxidant activity, Free Radical Scavenging Activity, Anti Radical Power, Reducing Power.

INTRODUCTION

Antioxidants interfere with the production of free radicals and are known to defuse them leading to limited risk of oxidative stress and associated disorders. They are the by-product of cell metabolism are involved in a variety of diseases and can cause damage to cellular bio-molecules like nucleic acid, proteins, lipids and carbohydrates and consequently may adversely affect immune functions (1-5). At cellular and molecular levels antioxidants inactivate free radicals and inhibit or delay oxidative processes by interrupting the radical chain reaction of lipid peroxidation (6-8). Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration (9). It has been found scientifically that the regular consumption of fruits, vegetables and whole grains, reduces the risk of chronic diseases associated with oxidative damage (10, 11). Phytochemicals like carotenoids, tocopherols, ascorbates, lipoic acids and polyphenols are strong natural antioxidants with free radical scavenging activity. Endogenous antioxidant enzymes like super oxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, minerals like Se, Mn, Cu, Zn, and certain vitamins exert synergistic actions in scavenging free radicals. Synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toulene (BHT) play a useful role in food and pharmaceutical industries (12-14). Polyphenols act against allergies, ulcers, tumors, platelet aggregation, cardiovascular diseases and can reduce the risk of cancer (15-18).

To find antioxidant potential of some under-utilized fruits, the extracts of their different parts were studied for their total phenolic content (TPC), antioxidant (AOA) and free radical scavenging activities (FRSA).

MATERIALS AND METHODS

Fruits collected from different natural habitats of the country were chopped, dried, powdered (40-mesh) and stored in polythene bags at 4° C. The powdered material (100 mg) was extracted with 50% MeOH : H_2O (1:1, 2 X 10 ml), overnight at room temperature. The Total phenolic content (TPC) in different extracts were measured by the method of Ragazzi and Veronese (19) 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 (20) and expressed as per cent inhibition relative to control.

Free radical scavenging activity (FRSA) of the extracts (1.0 mg/ml methanol) was assayed by using 1, 1-diphenyl-2-picryl- hydrazil (DPPH) radical (6×10^{-5} M in MeOH) according to Yen and Duh (21). The inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti radical power (ARP) was estimated as described by Kroyer (22). Reducing power of plant extracts (1.0 mg/ml in MeOH) was determined (23) by ferric reducing - antioxidant power assay and by using quercetin as 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 (24) and egg yolk (25). Ferrous ions chelating capacity was estimated as described by Decker and Welch (26). Ferric ion chelation was determined by the method of Wong & Kitts (27).

For HPLC analysis, 1 g of dried and powdered plant material was extracted with MeOH : H_2O (1:1, 1 X 20 ml) for 1 hour at room temperature. The plant extract was processed and subjected to HPLC (Shimadzu LC-10A Kyoto, Japan) for the qualitative and quantitative analysis (28). Results are the mean values of three replicates of the same sample and statistical analysis was performed by analysis of variance (ANOVA).

RESULTS

Phenols, a major group of phytochemicals has profound importance due to their antioxidant activity (AOA). In order to find their potential sources some fruits and their parts were studied for total phenolic contents (TPC), AOA and free radical scavenging activity (FRSA). They showed wide variation in TPC (Table 1) from 10.5 (*Carissa carandus*, fruit peel) to 343.2 mg/g (*Caesalpinia mexicana,* fruits). Fruit pericarp fibres of *Cocus nucifera* (135.0 mg/g); fruits pulp (120.4 mg/g) and seeds of *Emblica officinalis* (95.2 mg/g) were found to have high TPC. The fruits of *Acacia auriculiformis* (73.1 mg/g); fruit pulp (48.3 mg/g) and seeds (50.1 mg/g) of *Litchi chinensis,* seed kernel of *Magnifera indica* (59.6 mg/g) were with good amount of TPC. Total phenolic contents ranging from 2.12 to 69.4 g/100g in different parts of *Cassia fistula* had been reported (29). The AOA (Table 1) also showed a wide variation ranging from 20.3 (*Musa paradisiaca*, fruits) to 96.7% (*Caesalpinia mexicana,* fruits). The fruit fibres of *Cocus nucifera* (91.3 %); fruit pulp of *Emblica officinalis* (85.6 %) and *Acacia auriculiformis* (78.9 %) were found with high AOA. The seeds of *Emblica officinalis* (68.9%), fruit pericarp of *Aegle marmelos* (65.2%), fruit pericarp of *Castanopsis elegans* (54.3%), seeds of *Litchi chinensis* (50.1 %), fruit peel of *Malus sylvestris* (51.7 %) and *Mangifera indica* (54.8 %) were 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 (30). It was observed that plants with high phenols showed high AOA. On the other hand, fruit and fruit pericarp of *Aegle marmelos*, fruits of *Castanopsis elegans* and fruits of *Citrus sp.,* fruits of *Cyphomandra betaceae* were with low TPC and comparatively better AOA, probably due to the presence of anti-oxidants other than phenols. The high antioxidant activity of some samples with low phenolic content may be attributed due to some individual phenolic units with special high antioxidant activity or some other phyto-constituents (31). The presence of antioxidant enzymes (e.g., superoxide dismutase and catalase) and nonenzymatic antioxidants (e.g., anthocyananins, tocopherols, carotenoids, citrate, phosphate, and ascorbic acid) may also contribute to the overall observed antioxidative effect (32). The total phenols ranging from 2.12 to 69.4 g/100g and AOA 42.5 to 98.0% in different parts of *Cassia fistula*, *Cinnamomum zeylanicum* and *Moringa oleifera* had been reported (29, 33-35).

Plants	**Part**	**AOA %**	**TPC (mg GAE /g)**
Acacia auriculiformis	Fruits	78.9±0.4	73.1±1.3
Aegle marmelos	Fruit pulp	58.1±0.4	26.3±0.5
A. marmelos	Fruit pericarp	65.2±0.3	35.1±1.1
Ananas comosus	Fruit pulp	31.7±1.5	15.9±0.7
Averrhoa carambola	Fruits	37.3±9.7	19.7±0.3
Caesalpinia mexicana	Fruits	96.7±2.5	343.2±2.8
Capsicum annum	Fruits	39.7±1.5	17.7±0.9
Carica papaya	Fruit pulp	32.5±1.5	12.5±0.9
Carissa carandas	Fruits	39.1±1.7	14.8±0.3
C. carandas	Fruit peel	40.5±1.3	10.5±0.4
C. carandas	Fruit pulp	48.2±1.2	13.4±0.5
Castanopsis elegans	Fruits	54.3±1.0	16.9±0.7
Castanopsis tribuloides	Fruits	54.2 ±0.7	44.8±0.5
Citrulus colocynthus	Fruits	45.7±1.1	30.3±0.7
Citrus limon	Fruits	42.4±0.6	12.4±0.2
Citrus reticulata	Fruit pulp	46.6±1.4	11.9±0.4
Citrus sinensis	Pressed pulp	28.8±0.2	11.4±0.5
C. sinensis	Fruit peel	32.4±0.4	12.7±0.5
Cocos nucifera	Fruit pericarp fibres	91.3±1.2	135.0±2.7
Cucumis melo	Fruit pulp	44.8±0.6	19.1±0.7

Cucumis sativus	Fruits	26.7±0.2	16.1±0.1
Cyphomandra betaceae	Fruits	48.7±0.1	16.9±0.5
Eriolobus indica	Fruits	25.2±0.4	23.7±0.7
Elaeocarpus sikkimese	Fruits	28.7±1.0	18.2±0.4
Emblica officinalis	Fruits	85.6±1.3	120.4±2.1
E. officinalis	Seeds	68.9±0.7	95.2±1.3
Ficus hookeri	Fruits	29.4±1.5	20.7±0.9
Juglans regia	Fruits	39.3±1.2	19.9±0.4
Litchi chinensis	Fruit pulp	48.3±2.5	45.9±1.9
L . chinensis	seeds	50.1±1.4	57.9±1.0
L . chinensis	Fruit peel	43.9±1.6	42.4±1.2
Lycopersicon esculentum	Fruits	33.4±5.4	14.6±0.9
Malus sylvestris	Fruit peel	51.7±1.3	25.5±1.1
M. sylvestris	Fruits	42.8±0.9	17.2±0.4
Mangifera indica	Ripe fruit pulp	36.2±0.4	23.3±0.6
M. indica	Fruit peel	54.8±1.2	35.9±0.7
M. indica	Seed kernel	53.0±1.6	59.6±0.8
Musa paradisiaca	Fruits	20.3±1.3	12.1±0.4
M. paradisiaca	Fruit peel	23.8±1.7	11.3±0.6
CD at P<0.01		3.57	3.25

Table 1 Antioxidant activity (AOA %) assayed by auto-oxidation of β-carotene and linoleic acid and expressed as per cent inhibition relative to control and total phenolic contents (TPC) mg gallic acid equivalent (GAE) /g sample of some fruits (dry wt).

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 of *Caesalpinia mexicana,* fruit fibres of *Cocus nucifera* and fruit pulp of *Emblica officinalis* showed very low IC_{50} ranging from 0.009 to 0.016 mg/ml, low EC_{50} from 0.39 to 0.70 mg/mg DPPH, reasonably high values (142.1 to 256.3) of ARP. These samples also showed high reducing power as evident by their low 0.42 to 1.08 ASE/ml values. The fruits of *Caesalpinia mexicana* 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 (36). Fruit pulp, peel and seeds of *Litchi chinensis* with reasonable amount of phenols (Table 1) showed low ARP and ASE/ml (Table 2) in contrast the fruit pericarp of *Aegle marmelos* with comparatively lower phenols (35.1mg/g) exhibited good ARP (57.36) and reducing power (1.67 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 (30). The EC_{50} values 0.03 and 0.11 mg/ml in bark and leaves of *Azadirachta indica* and FRSA of *Trewia polycarpa* root extract had been reported (37, 38).

Fruits	**Parts**	**IC_{50}**	**EC_{50}**	**ARP**	**ASE/ml**
Acacia auriculiformis	Fruits	0.031	1.28	79.5	1.58
Aegle marmelos	Fruit pericarp	0.046	1.82	57.4	1.67
Caesalpinia mexicana	Fruits	0.009	0.39	256.3	0.42
Cocus nucifera	Fruit pericarp green fibres	0.015	0.65	153.8	1.08
Emblica officinalis	Fruits	0.016	0.70	142.1	0.63
E. officinalis	Seeds	0.038	1.65	60.6	1.32
Litchi chinensis	Fruit pulp	0.102	4.34	23.5	3.13
L. chinensis	seeds	0.068	2.95	33.8	2.62
L. chinensis	Fruit peel	0.060	2.61	38.3	2.18
Mangifera indica	Fruit peel	0.052	2.26	44.7	1.80
M. indica	Seed kernel	0.047	2.04	49.1	2.72
Quercetin	Standard	0.021	0.86	115.6	0.5
CD at P<0.01		0.053	0.46	0.67	0.29

Table 2 Free radical scavenging activity (FRSA) measured by using 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) in terms of IC50 = inhibitory concentration (mg/ml of extract); EC50 = efficiency concentration (mg/mg DPPH); ARP = anti radical power and reducing power (ASE/ml) of some promising fruits and their underutilized parts. ASE=Ascorbic acid equivalent.

Promising fruit samples were further subjected to concentration-dependent FRSA using different methods and expressed in terms of IC_{50} values. The IC_{50} values for inhibition of lipid peroxidation measured by ferric thiocyanate assay ranged from 0.21 to 3.13 mg/ml; fruits of *Caesalpinia mexicana* (0.21 mg/ml), fruit pericarp green fibres of *Cocus nucifera* (0.32 mg/ml); fruits (0.50 mg/ml) and seeds (0.92 mg/ml) of *Emblica officinalis* showed better inhibition of peroxide formation compared to reference standard, quercetin (1.27 mg/ml). Lipid peroxidation ability assayed by using egg yolk also exhibited similar results; however, the IC_{50} values indicate that most of the extracts inhibited lipid peroxidation at relatively lower concentration when assayed through ferric thiocyanate. The difference in percent inhibition in the above mentioned assays could be ascribed to several factors, including the indicators of different steps of lipid oxidation, polarity of polyphenolic compounds present in the extract and the antioxidative mechanisms exhibited by the compounds (39). The 40.35 and 56% inhibition of lipid peroxidation had been reported in water and ethanol extracts (2 mg/ml) of ginger respectively (40). The fruit and seed extracts of *Syzygium 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 in a concentration dependent manner (200-1000 mg/ml) (41). The iron reducing activity was determined in the selected extracts assayed through ferrous ion and ferric ion chelating capacity. The ferrous ion-chelating capacity of the promising fruits and their underutilized parts under study in terms of IC_{50} values varied from 0.12 (*Caesalpinia mexicana;* fruits) to 2.44 mg/ml (*Mangifera indica*, seed kernel). Further, the ferric ion chelating capacity of *Ceasalpinia mexicana* (0.22 mg/ml), green pericarp fibres of *Cocus nucifera* fruits (0.31 mg/ml) and fruits pulp of *Emblica officinalis* (0.29 mg/ml) was observed to be better compared to standard quercetin (0.65 mg/ml). Transition metal ions are known to catalyse the formation of free radicals. On the other hand, phenolic compounds can inhibit their formation by chelating with metal ions. Extracts of Holy basil (30) showed ferrous chelating capacity of 33.31 and 32.05% at a concentration of 0.75 and 1.0 mg/ml, while potato peel showed ferrous chelating capacity of 50% at 5.0 mg/ml (40).The ferrous ion chelating ability capacity of *Phaulopsis fascisepala* leaf extract increased with increasing concentration and reached 34.4% at a concentration of 2.0 mg /ml (42). In another study (27), the binding of Fe^{2+} by Butter Milk Solids (BMS) was 1.7 to 3.5 times greater than binding of Fe^{3+} at a concentration range of 1.0, 2.5, and 5 mM. All concentrations of BMS tested exhibited a similar affinity to bind both forms of iron.

Antioxidant effect of the polyphenols extracted from green tea leaves (43) and *Opuntia ficus-indica* (24) against oxidative DNA

damage showed protection against damage from free radicals. Polyphenols are potential protecting agent against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. Present studies together with the previous works suggest the triple synergistic action of phenols in scavenging ROS, repairing DNA and metal chelation (43).

Promising fruit samples were assayed for their specific phenolic composition (Table 3) through HPLC. The amount of caffeic acid varied from 48.5 to 2231.0 µg /g, chlorogenic acid 63.8 to 912.1 µg/g, ellagic acid 46.4 to 1429.1µg/g, ferulic acid 36.7 to 762.9 µg /g, gallic acid 181.6 to 2831.6 µg /g, protocatechuic acid 41.7 to 322.8 µg/g, and quercetin 44.6 to 367.6 µg /g. The presence of kaempferol was observed in fruits of *Acacia auriculiformis* and seeds of *Litchi sinensis* only whereas rutin was present in low quantities in fruits of *Caesalpinia Mexicana, Emblica officinalis* and fruit peel of *Litchi chinensis*. The fruit pulp of *Emblica officinalis* were found to be potential source of caffeic acid; fruits of *Caesalpinia mexicana* of ellagic acid, fruits of *Acacia auriculiformis* and *Caesalpinia mexicana* of gallic acid.

Fruits	Part	CA	CHL	EA	FA	GA	PCA	KMP	QC	RT
Acacia auriculiformis	Fruits	-	496.4	496.1	108.6	1484.6	322.8	191.36	86.1	-
Aegle marmelos	Fruit pericarp	--	136.8	248.5	98.3	873.6	47.9	--	56.9	--
Caesalpinia mexicana	Fruits	-	912.1	1429.1	36.7	2831.6	287.4	-	367.6	94.5
Cocus nucifera	Fruit pericarp green fibres	244.7	--	102.8	359.3	882.1	126.4	--	92.1	--
Emblica officinalis	Fruits	2231.0	540.0	--	463.2	546.7	--	--	126.9	54.2
Litchi chinensis	Fruit pulp	221.6	--	--	762.9	181.6	41.7	--	--	--
L. chinensis	seeds	--	--	46.4	--	--	70.1	9.3	--	--
L. chinensis	Fruit peel	96.2	71.4	-	112.5	247.1	82.6	-	57.4	24.8
Mangifera indica	Fruit peel	48.5	63.8	-	65.7	214.8	68.5	-	44.6	-
M. indica	Seed kernel	244.7	-	102.8	359.3	882.1	126.4	-	92.1	-

Table 3 Specific phenolic composition (µg/g sample) of some selected fruits and their under-utilized parts estimated through HPLC

GA= Gallic acid; CHA = Chlorogenic acid; CA = Caffeic acid, EA= Ellagic acid; FA=Ferulic acid; PCA= Protocatechuic acid; KMP = Kaempferol; QC = Quercetin; RT=Rutin

CONCLUSION

The fruits of *Caesalpinia mexicana, Acacia auriculiformis,* and fruit pericarp green fibres of *Cocus nucifera* were found to have high phenols and high AOA; low IC_{50}, low $EC_{50,}$ reasonably good values of ARP and strong free radical scavenging activity. The fruits of *Emblica officinalis* were found to be potential source of caffeic acid; fruits of *Caesalpinia mexicana* of ellagic acid, fruits of *Acacia auriculiformis* and *Caesalpinia mexicana* of gallic acid. The application of various methods used in present studies like lipid per oxidation, DPPH radical scavenging, and reducing power 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 biological activities.

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