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# Total phenol and antioxidant activity of some fruits and their under-utilized parts

*Prakash, D., [1]Upadhyay, G. and Gupta, C.

*Amity Institute for Herbal Research and Studies, Amity University-UP, Sector-125, Noida-201303, UP, India*
*[1]Department of Pomology, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

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**Abstract**

Some fruits and their under utilized parts were evaluated for total phenolic contents (TPC), antioxidant (AOA) and free radical scavenging activities (FRSA). The leaves (73.8 mg/g) and fruits of *Phoenix sylvestris*, fruits of *Ziziphus jujuba*, *Syzygium cumini* and *Zanthoxyllum acanthopodium* and leaves of *Protium serratum* were found to have high amounts of TPC (69.4 mg/g to 128.6 mg/g GAE). The inflorescence and leaves of *Phoenix sylvestris*, fruits of *Protium serratum*, *Syzygium cumini* and *Psidium guajava* were found with reasonably good AOA (60.7% to 84.9%). Plants with promising AOA were further investigated for FRSA using DPPH free radical assay in terms of inhibitory concentration ($IC_{50}$), efficiency concentration ($EC_{50}$), anti radical power (ARP) and reducing power (RP). Promising fruit samples were further subjected to concentration-dependent FRSA using different methods and expressed in terms of IC50 values. Fruit extracts (20 µg/ml) of *Phoenix sylvestris*, *Protium serratum* and leaves of *Zanthoxyllum acanthopodium* effectively prevented DNA nicking studied on pBR322 DNA. The fruits of *Passiflora edulis* were found to be good source of caffeic acid, fruits of *Phoenix sylvestris* of chlorogenic acid, *Phoenix sylvestris*, *Protium serratum* and leaves of *Zanthoxyllum acanthopodium* were rich in gallic acid. The identification of specific polyphenols was further substantiated by MS/MS analysis.



## Introduction

Free radicals are known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, as well as in aging processes. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage. Several epidemiological and in vitro studies have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems which is among the major causative factors in induction of many chronic and degenerative diseases. Synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes, however, they are not considered as safe for human health. The increased awareness of the health protecting properties of non-nutrient bio-active compounds found in fruits and vegetables, has directed immense attention to search antioxidants of natural origin (Nabavi *et al*., 2008; Prakash and Gupta, 2009; Prakash and Kumar, 2011).

Fruits and vegetables are good sources of natural antioxidants for the human diet, containing many different antioxidant components which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline in addition to other health benefits. These positive effects are believed to be attributed due to the presence of certain antioxidants like ascorbates, carotenoids, flavonoids, lycopene and polyphenols. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies. Phenolic compounds which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for the antioxidant capacity. Fruits offer protection against free radicals that damage lipids, proteins, enzymes 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

*Corresponding author.
Email: *dprakash_in@yahoo.com*

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 Kumar, 2011).

The objective of present studies was to determine the total phenolic contents, antioxidant and free radical scavenging activities of some fruits and their under utilized parts to evaluate their potential for use in nutraceuticals and functional foods.

## Materials and methods

*Fruit materials*

Healthy and disease free fruits including their underutilized parts were collected from different habitats 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 : $H_2O$ (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 distilled water. The absorbance of test mixture was measured at $\lambda_{max}$ 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 (1 mM = 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); 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 minutes 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 1N 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

Plant phenolics form a large group of natural compounds, ubiquitous in the plant kingdom. It is known that these secondary metabolites display a remarkable array of biochemical interactions, probably due to their antioxidant properties. These substances may act as potent metal chelators and/or free radical scavengers, however, it has been reported that the performance of these compounds in oxidative systems depends on activity–structure relationships (da Silva *et al*., 2007). In the present study, some fruits along with their under utilized parts were studied for total phenolic contents (TPC), antioxidant (AOA) and free radical scavenging activities (FRSA). The TPC showed wide variation (Table 1) from 6.1 (*Prunus persica*, fruits) to 136.5 mg/g GAE (*Phoenix sylvestris*, inflorescence). Leaves (73.8 mg/g) and fruits (128.6 mg/g) of *Phoenix sylvestris*, fruits of *Ziziphus jujuba* (106.8 mg/g) and *Syzygium cumini* ( 84.1 mg/g), fruits (97.2 mg/g) and leaves (69.4 mg/g GAE) of *Protium serratum* were found to have high 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 12.2% (*Prunus cerasus*, seeds) to 87.0% (*Phoenix sylvestris*, fruits). The inflorescence (80.5 %) and leaves (60.7%) of *Phoenix sylvestris*, fruits of *Protium serratum* (84.9%), *Syzygium cumini* (76.2%) and *Psidium guajava* (69.7%) 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%), 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, fruit peel (22.6 mg/g GAE) of *Prunus domestica* fruits (18.9 mg/g GAE) and fruit coat (17.1 mg/g GAE) of *Passiflora edulis* were with low TPC and comparatively better AOA exhibiting 58.4%, 67.1% and 42.9% respectively. 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). The presence of antioxidant enzymes like superoxide dismutase (SOD) and catalase and non enzymatic antioxidants such as anthocyanins, tocopherols, carotenoids 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 69.4 g/100 g 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).

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. Results are mean values of three replicates.

| Plants | Part | AOA (%) | TPC ( mg GAE /g) |
|---|---|---|---|
| *Castanopsis elegans* | Fruit | 28.5±2.9 | 13.1±3.2 |
| | Leaf | 31.8±1.7 | 16.0±2.5 |
| *Passiflora edulis* | Fruit | 67.4±4.6 | 18.9±2.6 |
| | Fruit Coat | 42.9±3.3 | 17.1±3.1 |
| *Psidium guajava* | Leaves | 46.5±2.3 | 37.9±1.3 |
| | Fruits | 65.4±4.2 | 40.4±1.0 |
| *Phoenix sylvestris* | Leaves | 62.7±2.4 | 73.8±2.4 |
| | Fruits | 87.0±3.2 | 128.6±2.7 |
| | Inflorescence | 80.5±1.3 | 136.5±6.5 |
| *Protium serratum* | Leaves | 55.6±1.8 | 69.4±2.6 |
| | Fruits | 84.9±4.7 | 97.2±4.1 |
| *Prunus armeniaca* | Leaves | 30.2±2.1 | 13.9±1.1 |
| | Fruit pulp | 33.7±2.9 | 12.5 ±1.0 |
| *Prunus cerasus* | Fruit pulp | 36.7±2.7 | 18.2±3.1 |
| | Seed | 12.2±4.2 | 8.7±2.5 |
| | Leaves | 19.8±3.2 | 12.6±2.1 |
| *Prunus domestica* | Fruit peel | 58.4±3.4 | 22.6±1.3 |
| | Fruit pulp | 24.8±2.4 | 29.1±1.4 |
| | Seeds | 25.8±3.1 | 17.3±0.6 |
| | Leaves | 31.2±1.3 | 16.9±1.4 |
| *Prunus persica* | Leaves | 51.2±2.6 | 19.3±1.1 |
| | Seeds | 16.4±1.0 | 14.9±1.2 |
| | Fruits | 19.4±1.1 | 6.1±0.1 |
| *Pyrenthrum communis* | Leaf | 53.0±2.5 | 28.7±2.2 |
| | Green Fruits | 41.2±3.4 | 45.1±3.1 |
| | Seeds | 32.1±1.7 | 37.9±2.5 |
| *Rubus ideaus* | Leaves | 19.6±4.3 | 10.9±5.1 |
| | Fruit coat | 23.3±2.9 | 10.5±1.7 |
| | Fruit | 21.2±4.6 | 10.0±3.8 |
| *Sterculia foetida* | Fruits | 40.8±2.1 | 29.1±3.6 |
| | Leaves | 60.2±2.3 | 51.4±3.7 |
| *Syzygium cumini* | Seed | 30.7±1.5 | 46.9±2.1 |
| | Fruit | 76.2±3.3 | 84.1±2.7 |
| | Leaves | 52.6±2.4 | 50.1±1.8 |
| *Zanthoxyllum acanthopodium* | Fruit | 54.1±3.9 | 68.6±4.1 |
| | Leaves | 49.5±3.2 | 34.5±3.5 |
| *Zizyphus jujuba* | Fruits | 69.7±4.3 | 106.8±3.1 |
| CD at P < 0.01 | - | 2.49 | 2.21 |

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 inflorescence of Phoenix sylvestris, fruits of *Protium serratum*, *Zizyphus jujuba* and *Psidium guajava* and leaves of *Zanthoxyllum acanthopodium* showed low $IC_{50}$ ranging from 0.021 to 0.044 mg/mg, low $EC_{50}$ from 0.92 to 1.91 mg/mg DPPH, reasonably high values (52.5 to 109.5) of ARP. They also showed high reducing power as evident by their low 0.47 to 1.51 ASE/ml values. The leaves

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/ml of extract); $EC_{50}$ = efficiency concentration (mg/mg DPPH); ARP = anti radical power and reducing power (ASE/ml) of some promising fruits and their under utilized parts.

| Plant | Parts | $IC_{50}$ | $EC_{50}$ | ARP | ASE/ml |
|---|---|---|---|---|---|
| *Castanopsis elegans* | Fruit | 0.190 | 8.26 | 12.1 | 0.81 |
| | Outer cover | 0.127 | 5.52 | 18.1 | 4.74 |
| *Passiflora edulis* | Fruit | 0.048 | 2.08 | 48.1 | 1.66 |
| | Outer cover | 0.104 | 4.52 | 22.1 | 3.21 |
| *Psidium guajava* | Fruit | 0.037 | 1.60 | 62.5 | 0.75 |
| | Leaves | 0.075 | 3.26 | 30.7 | 3.84 |
| *Prunus domestica* | Fruit pulp | 0.068 | 2.95 | 33.9 | 2.62 |
| | Fruit peel | 0.055 | 2.30 | 41.8 | 1.92 |
| | Seeds | 0.102 | 4.34 | 23.5 | 3.13 |
| | Leaves | 0.060 | 2.61 | 38.4 | 2.28 |
| *Phoenix sylvestris* | Fruit | 0.021 | 0.92 | 109.5 | 1.14 |
| | Seed | 0.128 | 5.56 | 17.9 | 4.10 |
| | Inflorescence | 0.030 | 1.30 | 76.9 | 2.33 |
| *Protium serratum* | Fruit | 0.029 | 1.26 | 79.4 | 1.74 |
| | Leaves | 0.052 | 2.26 | 44.2 | 1.80 |
| *Sterculia foetida* | Fruits | 0.065 | 2.82 | 35.4 | 1.51 |
| | Leaves | 0.075 | 3.26 | 30.7 | 4.84 |
| *Syzygium cumini* | Fruit | 0.068 | 2.95 | 33.9 | 1.72 |
| | Seed | 0.231 | 10.0 | 10.0 | 4.27 |
| *Zanthoxyllum* | Fruit | 0.081 | 3.52 | 28.4 | 2.51 |
| *acanthopodium* | Leaves | 0.039 | 1.69 | 59.2 | 0.47 |
| *Zizyphus jujuba* | Fruits | 0.044 | 1.91 | 52.5 | 1.53 |

of *Zanthoxyllum acanthopodium* 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). 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 dependent on the method of analysis, biodiversity, genetic, seasonal and geographical variations (Kim *et al.*, 2006; Prakash *et al*., 2012).

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, and therefore, 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 ranged from 0.43 to 4.51 mg/ml. The fruits of *Phoenix sylvestris* (0.43 mg/ml), fruits of *Protium serratum* (1.17 mg ml) and leaves of *Zanthoxyllum acanthopodium* (1.09 mg/ml) showed better inhibition of peroxide formation compared to reference standard

Table 3. Free radical scavenging activity assayed by different methods expressed as $IC_{50}$ (mg/ml) in some promising fruits and their under utilized parts 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 = Non-site specific inhibition of hydroxyl radical-mediated deoxyribose degradation; F = Site-specific inhibition of hydroxyl radical-mediated deoxyribose degradation;
Inflo = Inflorescence.

| Plants | Parts | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|
| *Phoenix sylvestris* | Fruit | 0.43 | 0.32 | 0.40 | 1.75 | 0.31 | 0.80 |
| | Seed | 3.21 | 3.15 | 2.12 | 4.06 | 2.97 | 2.94 |
| | Inflo | 1.40 | 0.94 | 1.27 | 1.92 | 1.74 | 1.80 |
| *Passiflora edulis* | Fruit | 2.24 | 2.40 | 2.78 | 2.24 | 3.25 | 4.12 |
| | Fruit coat | 4.51 | 1.82 | 2.53 | 4.87 | 2.50 | 2.14 |
| *Protium serratum* | Fruit | 1.17 | 0.58 | 0.62 | 2.44 | 1.10 | 0.92 |
| | Leaves | 4.30 | 2.53 | 3.75 | 2.96 | 2.07 | 3.46 |
| *Zanthoxyllum* | Fruit | 1.96 | 2.24 | 2.09 | 3.63 | 4.02 | 3.14 |
| *acanthopodium* | Leaves | 1.09 | 1.60 | 1.36 | 3.14 | 2.71 | 1.62 |
| *Zizyphus jujuba* | Fruits | 1.28 | 1.25 | 1.66 | 1.50 | 2.51 | 2.17 |
| Quercetin | | 1.27 | 0.52 | 0.66 | 1.85 | 1.06 | 0.58 |
| LSD at $P < 0.01$ | | 2.16 | 1.53 | 1.79 | 2.86 | 2.52 | 2.01 |

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 µg/ml (Banerjee *et al*., 2005).

The ferrous ion-chelating capacity (Table 3) in terms of $IC_{50}$ values varied from 0.32 (*Phoenix sylvestris*, fruits) to 3.24 mg/ml (*P. sylvestris*, seeds). Further, the ferric ion chelating capacity of fruits of *P. sylvestris* (0.47 mg/ml) and *Protium serratum* (0.62 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 *Zizyphus jujuba* (1.50 mg/ml), fruits (1.75 mg/ml) and inflorescence (1.92 mg/ml) of *Phoenix sylvestris* 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 *Phoenix sylvestris* and *Protium serratum* 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 0.80 (*Phoenix sylvestris*, fruits) to 4.12 mg/ml (*Passiflora edulis*, fruit coat). 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 (Figure 1) that showed significant reduction in the formation of nicked DNA and increase in native DNA (super coiled). Fruit extracts (20 µg/ml) of *Phoenix sylvestris*, *Protium serratum* and leaves of *Zanthoxyllum acanthopodium* effectively prevented DNA nicking and mitigated the oxidative stresses on susceptible biomolecules. The protection offered by the fruit extracts of *Phoenix sylvestris*, (lane 6) and *Protium serratum* (lane 7) 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; Prakash *et al.*, 2012).

Table 4. Specific phenolic composition (µg/g dry weight) of some selected fruits and their underutilized parts estimated through HPLC

| Plant | Part | CA | ChA | EA | FA | GA | PCA | KMP | QC | RT |
|---|---|---|---|---|---|---|---|---|---|---|
| *Protium serratum* | Fruit | 44.3 | - | 53.9 | 646.2 | 454.2 | - | 245.9 | - | 84.4 |
| | Leaves | 32.7 | 137.9 | - | 203.9 | 179.5 | - | 87.2 | - | - |
| *Phoenix sylvestris* | Inflo | 13.2 | 45.4 | - | 59.14 | - | 24.7 | 23.5 | - | - |
| | Fruits | - | 587.3 | 41.6 | | 993.7 | - | - | 186.6 | 94.6 |
| *Passiflora edulis* | Fruit coat | 24.2 | 194.1 | - | - | - | 96.2 | - | 72.3 | - |
| | Fruit | 670.1 | 122.0 | - | 32.4 | 125.0 | 45.1 | - | 41.0 | 35.1 |
| *Ziziphus jujuba* | Fruits | 139.4 | - | 131.8 | 114.6 | 785.1 | 88.4 | - | 75.9 | 166.1 |
| *Zanthoxyllum* | Fruits | 43.7 | - | - | 39.4 | 324.8 | - | 193.2 | 374.2 | - |
| *acanthopodium* | Leaves | 32.7 | 35.9 | - | 427.1 | 638.7 | 21.6 | - | 86.6 | 56.3 |

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

Table 5. Specific phenolic composition of some fruits and their underutilized parts identified by LC-MS/MS

| Phenols | Plants | Ion full scan MS | MS/MS approach |
|---|---|---|---|
| | | [M-H]- | Fragments | Product ion scan |
| Caffeic acid | A, B, C, E, F, I, J, H | 179 | 135 | 179 |
| Chlorogenic acid | B, C, D, E, F, I | 353 | 191 | 353 |
| Ellagic acid | A, D, G | 301 | 170, 125 | 170 |
| Ferulic acid | A, B, C, F, G, H, I | 193 | 178, 149 | 193 |
| Gallic acid | A, B, D, F, G, H, I | 169 | 125 | 169 |
| Protocatechuic acid | C, E, F, G, I | 153 | 109 | 153 |
| Kaempferol | A, B, C, H | 285 | 133, 151 | 285 |
| Quercetin | D, E, F, G, H, I | 301 | 151 | 301 |
| Rutin | A, D, F, G, H, I | 609 | 301 | 609 |

A = *Protium serratum* (fruits), B = *Protium serratum* (leaves), C = *Phoenix sylvestris* (Inflorescence), D = *Phoenix sylvestris* (fruits), E = *Passiflora edulis* (fruit coat), F = *Passiflora edulis* (Fruit), G = *Ziziphus jujuba* (fruits), H= *Zanthoxyllum acanthopodium* (fruits); I = *Zanthoxyllum acanthopodium* (leaves).

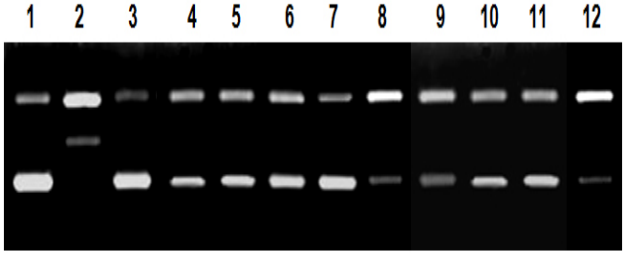


Figure 1. Concentration dependant inhibitory effects of extracts on pBR 322 DNA nicking caused by hydroxyl radicals. Fenton = Fenton's reagent.

Lane 1: pBR322 DNA; Lane 2: DNA + Fenton; Lane 3: DNA + Fenton + Catalase (2 units); Lane 4: DNA + Fenton + *Passiflora edulis* (Fruit); Lane 5: DNA + Fenton + *Ziziphus jujuba* (fruit); Lane 6: DNA + Fenton + *Protium serratum* (fruits); Lane 7: DNA + Fenton + *Phoenix sylvestris* (fruits); Lane 8: DNA + Fenton + *Zanthoxyllum acanthopodium* (fruit) ; Lane 9: DNA + Fenton + *Phoenix sylvestris* (Inflorescence); Lane 10: DNA + Fenton + *Protium serratum* (leaves); Lane 11: DNA + Fenton + *Zanthoxyllum acanthopodium* (leaves); Lane 12: DNA + Fenton + *Passiflora edulis* (Fruit Coat).

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 13.2 to 670.1 µg/g, chlorogenic acid 35.9 to 587.3 µg/g, ellagic acid 41.6 to 131.8, ferulic

acid 32.4 to 646.2 µg/g, gallic acid 125.0 to 993.7 µg/g and quercetin 41.0 to 374.2 µg/g. The presence of kaempferol was observed in fruits and leaves of *Phoenix sylvestris* and fruits of *Protium serratum* and *Zanthoxyllum acanthopodium* only whereas rutin was present in close variation of 35.2 to 166.1 µg/g. Ellagic acid was found to be present only in fruits of *Phoenix sylvestris*, *Protium serratum* and *Zizyphus jujuba* µg/g. The fruits of *Passiflora edulis* were found to be good source of caffeic acid, fruits of *Phoenix sylvestris* of chlorogenic acid, fruits of *Phoenix sylvestris*, *Protium serratum* and leaves of *Zanthoxyllum acanthopodium* of gallic acid. 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; Prakash *et al*., 2012).

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; Prakash *et al*., 2011).

## Conclusions

The foregoing study suggests that the fruits of *Phoenix sylvestris* and *Protium serratum* 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 in free radical generated DNA damage indicating the strong free radical scavenging activity. The fruits of *Passiflora edulis* were found to be good source of caffeic acid, fruits of *Phoenix sylvestris* of chlorogenic acid, fruits of *Phoenix sylvestris*, *Protium serratum* and leaves of *Zanthoxyllum acanthopodium* of gallic acid. The application of various methods in present studies like lipid per oxidation, DPPH radical scavenging, reducing power, metal chelating capacity and DNA nicking to evaluate AOA at multiple concentration followed by studies on their specific phenolic composition were conducted to draw a justified conclusion.

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