Antioxidant Potential of the Polyphenolics in
Grewia asiatica, *Eugenia jambolana* and *Carissa carandas*

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Abstract

The fruits *G. asiatica*, *E. jambolana* and *C. carandas* were extracted with methanol and the extracts were partitioned into four polyphenolic fractions: Flavanols, flavonols, phenolic acids and anthocyanins using solid phase extraction. Each fraction was then analyzed for total phenolics, flavonoids, antioxidant activity by DPPH, β-carotene-linoleic acid assay and total reducing power. Total phenolics ranged 6.64-107, 56.20-398, 33.38-315 and 20-201 mg/100 g in phenolic acid, flavanol, flavonol and anthocyanin fractions respectively of these fruits which explained the order of their antioxidant activity and reducing power as flavanols > flavonols > anthocyanins > phenolic acid. Total phenolics were highest in *E. jambolana* fractions (107-398 mg/100 g) but the maximum antioxidant activity (62-85% in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 58-89% in β-carotene-linoleic acid assay) was shown by the fractions in *G.asiatica* (total phenolics, 67-151 mg/100 g). At all concentrations, the DPPH scavenging activity of flavanol fraction in G. *asiatica* (85% at 20 ppm) was comparable to BHA (89%).

Keywords: *Grewia asiatica*, *Eugenia jambolana*, *Carissa carandas*, polyphenols, antioxidant activity

1. Introduction

The over production of active oxygen species like O_2, H_2O_2 and OH^- may lead to tissue injury (Thomas, 1995), DNA damage, thiol oxidation and lipid peroxidation (Halliwell et al., 1995), cardiac disorder, chronic gut inflammation, cancer and AIDS in humans (Halliwell et a1., 1992). An antioxidant is known to delay or prevent oxidation of substrate (Halliwell, 1990). The role of polyphenols as radical scavengers and in increasing the resistance of LDL oxidation involved in heart diseases (Rice-Evans et al., 1995) have been demonstrated by many *in vitro* studies. Flavonoids, a family of polyphenolic compounds, are widely distributed pigments, possessing anti-radical and chelating properties. They can scavenge free hydroxyl and peroxy radicals or may extract iron ions to depress superoxide-driven Fenton reaction (Afanas'ev et al., 1989). It is established that antioxidant potential of lots of fruits is based on their flavonoid contents (Wang et al., 1996; Heinonen et al., 1998) and polyphenolics in foods are more efficient antioxidants than vitamins C & E, and β-carotene (Vinson et al., 1995).

There are various methods to assess the *in vitro* antioxidant potential of isolated compounds involving different mechanisms, for instance, scavenging of reactive oxygen species or reactive nitrogen species like peroxynitrite, the OH radical and superoxide, measuring the vanishing of free radicals such as $ABTS^+$ (2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonate) cation radical) or DPPH (2,2-diphenyl-1-picrylhydrazyl) and determining the total antioxidant power like ferric reducing/antioxidant power. A number of techniques may be used for measuring differences between the antioxidant potential of the compounds. Since most of the compounds tested are multifunctional, therefore it is necessary to measure more than one property (Frankel & Meyer, 2000). Thus the results of antioxidant capacity rely on the procedure used and a single method may not be sufficient enough to predict antioxidant efficacy of the various compounds or extracts. Studies have been

performed to evaluate the role of *Grewia asiatica, Eugenia jambolana* and *Carissa carandas* extracts in different chemical *in vitro* models. None of the studies have reported the antioxidant activities associated to the various polyphenolic fractions of G. *asiatica* and *C. carandas* separated from the crude extracts on the basis of their chemistry. Several studies have reported the isolation of polyphenolic compounds with their respective antioxidant capacities in *E. jambolana* but the way the fractionation has been performed, types of the fractions separated on the basis of their chemical nature and the correlation of total phenolics, flavonoids, anthocyanins in each fraction with their antioxidant activities presented in this study is quite different and new.

2. Materials and Methods

2.1 Sampling

Sample fruits – *G. asiatica* and *E. jambolana* – were purchased once in a size of 10 kg each in May and June 2009 respectively. About 12 kg of *C. carandas* fruits were collected in July from Karachi University campus Sample fruits – being very perishable in nature and sold within 1-2 days after harvesting, were stored immediately at -5°C after washing and packing.

2.2 Extraction and Fractionation of Polyphenolics

Extraction, fractionation of polyphenols into anthocyanins (Fraction II) and non anthocyanins and further division of non anthocyanin fraction into flavanols (Fraction Ia), flavonols (Fraction Ib) and phenolic acids (Fraction Ic) were performed as described by Siddiqi et al. (2011).

2.3 Quantitative Analyses of the Fractions Ia, Ib, Ic and II

All the fractions were analysed in triplicate for total phenols and flavonoids by the method of Jayaprakasha et al. (2003) and Zhishen et al. (1999) respectively and the data were expressed as mean ± standard error. Fraction II was also analysed spectrophotometrically by the pH differential method for total anthocyanins (Wrolstad et al., 2005).

2.4 Antioxidative Potential

Antioxidant activities of all the extracts were determined by three different methods- DPPH radical scavenging system (Miliauskas et al., 2004), β-carotene-linoleic acid assay (Skerget et al., 2005) and ferric reducing assay (Jayaprakasha et al., 2001).

2.5 Statistical Analyses

All of the experiments were performed in triplicate, and the data were expressed as the mean ± SEM. Differences in antioxidant activity of the fractions were analyzed for significance using one-way analysis of variation (ANOVA) followed by a Bonferroni test for multiple comparisons using Originlab 8.0.

3. Results and Discussion

All the fractions showed potent radical-scavenging activity. The results of the activity of the four fractions and butylated hydroxyl anisole (BHA) are shown in Table 1. The radical scavenging activity of the fractions varied from 61-85% for *G. asitica*, 50-78% for *E. jambolana* and 41-70% for *C. carandas* at 20 ppm ($p < 0.05$). The maximum activity was noted in *G. asiatica* fractions and the minimum in *E. jambolana* fractions. In all fractions the activity increased significantly with concentration ($p < 0.05$). Though the total phenolics and flavonoids were higher in *E. jambolana*, the antioxidant activities of *G. asiatica* fractions were relatively higher ($p < 0.05$). The order of antioxidant activity of the fractions was corresponding to their order of phenolic and flavonoid contents i.e., Fraction Ib > Fraction Ic > Fraction II > Fraction Ia (Table 2) in all three fruits. At all concentrations, the activity of Fraction Ib from *G. asitica* was comparable to BHA. Fraction Ib of *E. jambolana* and *C. carandas* also showed good radical scavenging activity.

Table 1. Rdical scavenging activities of the fractions derived from *G. asiatica*, *E. jambolana* and *C.carandas* by DPPH method

Fruit Sample	Fractions	Concentration of fractions			
		5 ppm	10 ppm	15 ppm	20 ppm
G. asiatica	Fraction Ia	24±0.23	38±1.60	48±0.8	62±0.67
	Fraction II	30±0.89	50±0.98	58±0.67	70±0.98
	Fraction Ic	39±1.20	58±1.30	62±1.45	78±1.23
	Fraction Ib	48±2.30	62±1.56	75±1.89	85±3.23
E. jambolana	Fraction Ia	15±0.67	30±0.23	41±0.56	50±2.03
	Fraction Ib	25±0.34	45±0.34	50±0.45	67±1.20
	Fraction Ic	31±0.20	50±0.56	58±0.32	68±2.30
	Fraction II	42±0.17	55±0.23	68±0.23	78±1.45
C. carandas	Fraction Ia	10±1.23	27±0.56	35±0.23	41±0.56
	Fraction Ib	21±0.78	33±0.34	48±0.45	62±1.20
	Fraction Ic	28±0.96	42±0.23	51±0.56	65±2.30
	Fraction II	40±0.23	50±0.12	61±0.78	70±1.45
BHA		52±3.20	68±1.23	78±2.6	89±3.50

Means ± SEM of triplicate assays. For a given fruit sample, values in the same column and row were significantly different at $p < 0.05$.

Table 2. Anthocyanin, flavonoid and total phenolic contents (data expressed as milligrams per 100 g of weight) (Siddiqi et al., 2011)

Fruit Sample	Fractions	Total phenolics[a]	Flavonoids[b]	Anthocyanins[c]
G. asiatica	Fraction Ia	67±0.98	-	-
	Fraction Ib	288±0.07	178±0.23	-
	Fraction Ic	222±1.90	165±0.23	-
	Fraction II	151±0.40	100±0.90	72±6.0
E. jambolana	Fraction Ia	107±0.20	-	-
	Fraction Ib	398±0.21	287±0.24	-
	Fraction Ic	315±0.41	256±1.30	-
	Fraction II	201±0.51	151±0.34	13±0.31
C. carandas	Fraction Ia	6.64±0.14	-	-
	Fraction Ib	56.20±0.2	36±0.092	-
	Fraction Ic	33.38±0.4	17.0±0.01	-
	Fraction II	20±0.210	9±0.660	6.45±0.36

(-) estimation not performed

Means ± SEM of triplicate assays. Values in the same column were significantly different at $p<0.05$
[a]Concentration based upon gallic as standard. [b]Concentration based upon catechin as standard. [c]Concentration based upon cyanidin-3-glucoside as standard.

Table 3. Antioxidant activities of the *G. asiatica*, *E. jambolana* and *C. carandas* fractions using β-carotene-linoleic acid assay

	% Inhibition		
	G.asiatica	*E.jumbolana*	*C.carandas*
Fraction Ia	58±1.23	50±0.98	39±1.45
Fraction II	65±2.30	55±3.10	48±2.09
Fraction Ic	75±1.87	65±1.34	52±1.65
Fraction Ib	89±2.00	80±2.09	61±0.98

Values are represented as mean ± standard error. Values in the same column and row were significantly different at $p < 0.05$

All fractions of the fruits effectively inhibited the oxidation of β-carotene in the linolenic emulsion system ($p < 0.05$) (Table 3). Like DPPH, the highest inhibition of β-carotene oxidation was shown by *G. asiatica* fractions (58-89%), while the lowest inhibition was shown by *C. carandas* fractions (39-61%). DPPH IC_{50} values of the successive extracts of *G. asiatica* leaves were found to be 249.60 ± 7.37, 16.19 ± 2.132, 26.17 ± 1.49, 27.38 ± 1.80, 176.14 ± 5.53 and 56.40 ± 3.98 µg/mL by Gupta et al. (2007). Our results show that the DPPH IC_{50} values ranges between 5-20 ppm for *G. asiatica* fractions (Table1). Comparison of these results indicates that *G. asiatica* fruit has much more antioxidative potential than *G. asiatica* leaves. DPPH IC_{50} of 8.6 µg/mL for *E. jambolana* fruit (at 192.3 mg GAE/g total phenols) reported by Hossain et al. (2008) and 78.2% for fruit peel anthocyanins reported by Veigas (2007) at 2.5 ppm are lower than our values which ranges 5-10 ppm for almost all the fractions (Table 1). The antioxidant activity is dependent upon the reducing ability (Tanaka et al., 1988). Table 4 shows the reducing power of the fractions using potassium ferricyanide reduction method. At 50 ppm concentration, the absorbance at 700 nm ranged 1.56-3.1, 1.2-2.44 and 0.67-1.92 for *G. asiatica*, *E. jambolana* and *C. carandas* respectively.

Table 4. Reducing power of the fractions derived from *G. asiatica, E. jambolana* and *C.carandas*.

	Concentration	*G.asiatica*	*E.jumbolana*	*C.carandas*
	5 ppm	0.52±0.09[1a]	0.50±0.13[1a]	0.35±0.05
Fraction Ia	10 ppm	0.75±0.05	0.65±0.03	0.41±0.05
	25 ppm	1.30±0.10	0.80±0.02	0.48±0.05
	50 ppm	1.53±0.06	1.25±0.04	0.70±0.09
	5 ppm	0.80±0.02	0.65±0.10	0.60±0.10[1c]
	10 ppm	1.00±0.04[1b]	0.90±0.07[1b]	0.50±0.06[1c]
Fraction II	25 ppm	1.60±0.10	1.45±0.10	0.61±0.05[1c]
	50 ppm	2.00±0.12	1.75±0.04	1.10±0.09
	5 ppm	0.85±0.09[1d]	0.80±0.04[1d]	0.70±0.08[1d]
	10 ppm	1.40±0.13	1.10±0.09	0.60±0.09[1d]
Fraction Ic	25 ppm	2.00±0.05	1.75±0.10	1.00±0.06
	50 ppm	2.60±0.10	2.10±0.17	1.70±0.12
	5 ppm	1.00±0.05	0.85±0.07[1e]	0.82±0.04[1e]
	10 ppm	1.80±0.06	1.25±0.08	0.90±0.08
Fraction Ib	25 ppm	2.40±0.14	2.00±0.12	1.38±0.06
	50 ppm	3.10±0.05	2.55±0.11	1.86±0.13

Values are represented as mean ± standard error. Values in the same column and row were significantly different at $p < 0.05$ except where the same superscripts have been used to show no significant difference.

The reducing ability of *G. asiatica* was greater than that of *E. jambolana* and *C. carandas* ($p < 0.05$). The reducing property relates to the presence of reductones (Pin-Der, 1998) which not only break the free radical sequence by providing a single hydrogen but also quench peroxide formation by reacting with precursors of peroxide (Gordon, 1990). The results show that the evident antioxidant activity of the fractions from these three fruits were the consequence of their reducing potential and the ability to inhibit free radicals. The order of antioxidant activity of the fractions (Fraction Ib > Fraction Ic > Fraction II > Fraction Ia) can be explained on the basis of total phenolic contents and the relationship between structure and antioxidant activity of flavonoids derived from the literature. Total phenolics were least in the Fraction Ia (Table 2). Relative antioxidant activity of phenolic acids and their esters is dependent on the number of hydroxyl groups in the molecule. The H-donating ability of hydroxy benzoates is affected due to steric hindrance (Stern et al., 1996, Rice-Evans et al., 1996). Thus because of a single carboxyl group, monohydroxy benzoic acid is weak compared to dihydroxy and trihydroxy benzoic acids in which the activity also depends on the relative positions of the hydroxyl groups (Rice-Evans et al., 1996). In Fractions Ia of the three fruits, the phenolic acids identified include vanillic, iso-vanillic, ferulic, iso-ferulic and gallic acid. All of these possess monophenolic ring and hence are not effective hydrogen donors. Thus the antioxidant activity of the Fraction Ia (phenolic acid fraction) was low. The second least activity of Fraction II of the fruits is possibly due to the glycosylation of the anthocyanins in the fractions. Hopia and Heinonen (1999) indicated that glycosylation of flavonoids at position 3 decreases their activity compared to their aglycones while Shahidi and Wanasundara (1992) and Kapiotis et al. (1997) demonstated that a saturated heterocyclic ring with 7-glycosylsation and a single hydroxyl group on the B ring suppresses the antioxidant activity. The radical scavenging activity of procyanidins is much higher than flavonoid monomers (Hagerman et al., 1998), therefore tannins are more powerful scavengers than flavonoids and phenolic acids. Large molecules are effective scavengers compared to small molecules. Ellagitannins are less active than gallotannins of corresponding size (Yokozawa et al., 1998). This explains the highest antioxidant activity of Fraction Ib (polymeric flavanols). The maximum activity of the flavonols is derived from the fact that 3-OH group is attached to the 2,3-double bond and is adjacent to the carbonyl at position 4 in the C ring (Shahidi & Wanasundara, 1992) which explains comparatively higher antioxidant capacity of Fraction Ic (flavonols).

Acknowledgements

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