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Evaluation of antioxidant activity, total phenolics and phytochemical content of selected varieties of karonda fruits (*Carissa carandas*)

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ABSTRACT

Karonda (*Carissa carandas* L.) is a hardy, drought tolerant, low maintenance shrub, whose berries under-utilized but have potential to be promoted in the wastelands of India, and thus be of succour to farmers. The fruits are astringent due to high pectin content and therefore popular only as preserves. In this study the total phenol, flavonoid and anthocyanin contents and their individual components in three promising table collections of karonda: variety Konkan Bold, and promising collections CHES K-II/7 and CHES K-V/8 compared, and correlated with the antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and Ferric reducing antioxidant potential. The results indicated that these karonda collections are moderately good sources of phenols, flavonols and anthocyanins, the amounts of phenols and flavonoids correlated positively with the antioxidant activity. The phytochemical profile of individual components of the phenolic acids, revealed high concentrations of vanillic, protocatechuic, *t*-cinnamic, ferulic, chlorogenic, 2,4-dihydroxy benzoic, syringic and salicylic acids; flavonoids rutin, myricetin and umbelliferone; and glucosides of the anthocyanins cyanidin and pelargonidin. Further, CHES K-V/8 and CHES K-II/7 in addition to having favourable horticultural traits was superior to Konkan Bold, and may be recommended for release as varieties with health promoting polyphenols and antioxidant activity.

Key words: Antioxidants, Functional food, HPLC-MS, Karonda, Phenolics

Karonda (*Carissa carandas* L.) also called 'Christ Thorn Tree', belongs to the family *Elaeocarpaceae*. Karonda is an evergreen shrub, dichotomously branched, with short stem and strong thorns. It is a hardy, drought tolerant plant of the dryland, growing in a wide range of soil and climatic conditions, suited to waste lands and also thriving well as a rainfed crop, a succour to tribal communities in various parts of India - Bihar, West Bengal, Uttar Pradesh and South India (Banik *et al.* 2012). Karonda flowers in March-April which continues up to November in some parts of eastern India. About 120–130 days are required from fruit set to maturity and each tree produces 4 to 5 kg fruits. Because of its soft flesh and high moisture content, the storage life of karonda is very short - a week at 13 °C and 95% relative humidity (Mitra *et al.* 2010). It is also grown in South Africa, Australia, Malaysia, Sri Lanka, Bangladesh and Myanmar. The details of area and production of karonda in India are not available, as the shrubs grow wild and no systematic cultivation is undertaken.

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Formerly two distinct varieties: *C. Carandas* var. amara with oval, dark-purple, red-fleshed fruits, of acid flavour; and *C. carandas* var. dulcis with round, maroon fruits, with pink flesh and sweet-sub-acid flavour were identified. These and other variations throughout seedling populations (Morton 1987). In India, three promising plants were reported by Bhagwat (1984) at the Konkan Krishi Vidyapeeth (KKV), Dapoli, based on fruit size and color. Kumar and Singh (1993) also identified four types of fruits, viz. green, white with pink blush, green with purple blush and maroon in eastern Uttar Pradesh, India. Crop improvement exercises at ICAR-Indian Institute of Horticultural Research Regional Station at Chettalli, Coorg, have yielded elite table varieties of karonda (Anonymous 2014-15). Average fruit weight ranges from 1.6 to 4.7 g and average number of seeds/fruit from 5 to 11; moderate variation was observed in the biochemical composition of the fruit, with ascorbic acid content ranging from 10.26 to 17.94 mg/100 g, reducing sugars from 0.93 to 2.4%, non-reducing sugars from 0.57 to 1.33% and total soluble solids from 3 to 4.5%.

Karonda fruits are sour and astringent, acidic to sweet in taste with a peculiar aroma, and are not popular as a fresh fruit due to its high pectin content. Ripe fruits find use in the processing industry for the preparation of preserves (Bose *et al.* 1999). The fruits are a rich source of iron and vitamin C, and thus have antiscorbutic properties and are useful in

prevention of anaemia. Karonda is reportedly useful in curing stomachache and is anthelmintic (Das *et al.* 2013). The root extracts are used in lumbago, chest complaints and venereal diseases. In Ayurveda, the unripe fruits are used as astringent, appetizer, antipyretic, antidiabetic (Iyer and Dubhash 2006, Itankar *et al.* 2011). It is used by tribal healers in the Western Ghats region of Kodagu in Karnataka in traditional systems of medicine. The above information and some literature on proximate composition and medicinal importance is all that is available on karonda fruits.

As aerobic organisms, the production of potentially dangerous free radicals as by-products of respiration is inescapable. It is generally understood that oxidative stress is a major player in the pathophysiology of a number of diseases and debilities (de Groot and Rauen 1998). Among the protective bioactive constituents in fruits and vegetables, polyphenols have been reported to be the most crucial for human health (Scalbert and Williamson 2000). Though our body has its repertoire of antioxidants, supplementation with dietary antioxidants is reported to protect from oxidative damage (Karakaya 2004). Phenolic compounds, the largest and most diverse class of secondary metabolites in plants, are effective antioxidants (Kondratyuk and Pezzuto 2004). Consumption of polyphenol rich food, as borne out by the benefits of a Mediterranean diet and the famous 'French Paradox' are proof of this concept (Koren *et al.* 2010). As information on the phenols, flavonoids and anthocyanins and the antioxidant properties of under-utilized fruit crops especially karonda is lacking, this study was initiated with an objective to estimate and profile these secondary metabolites, and to measure the antioxidant potential in three selected table collections of karonda, Konkan Bold, CHES K-II-7 and CHES K-V/8.

MATERIALS AND METHODS

The experimental plot of karonda is located in the Central Horticultural Experiment Station (CHES), Chettalli, Karnataka (12°26' N latitude and 75°57' E longitude at 1050 m above sea level). The soil is a deep, dark brown, well drained sandy loam to sandy clay loam with 28.5% clay content and pH of 5.70. The weather conditions in CHES, Chettalli, during the time of harvest, June-July 2013 and 2014 are given in Table 1 and 2.

This study was carried out in 2013 to 2015, as part of long term ongoing experiment where the proximate composition of a few under-utilized fruits are being profiled, as we currently have no data pertaining to our collections. The data if available on the nutrient composition of karonda among other underutilized crops is either outdated, or largely based on work abroad and compiled by the National Institute of Nutrition (Gopalan *et al.* 2009).

After extensive survey and evaluation **o**f 40 karonda accessions**,** three promising lines of karonda were shortlisted based on preliminary evaluation: Konkan Bold, CHES K-II-7 and CHES K-V/8. These were analyzed for phenolics, flavonoids, anthocyanins and antioxidant potential. Konkan Bold (Fig 1a), one of the first varieties identified as table variety has bold and attractively dark coloured fruits with an ideal balance of sweetness and acidity, and high yield. CHES has registered another promising collection CHES K-V/8 (Fig 1b), with the National Bureau of Plant Genetic Resources, New Delhi, also a promising juicy and sweet table purpose variety, with an attractive dark colour and less acidity; even if the yield is only half that of Konkan Bold. CHES K-II-7 (Fig 1c) is yet another promising collection with thin skin, dark red, firm, juicy and sweet pulp, suitable for table and processing purposes; it had the highest pulp content and least acidity of the three collections (Table 3).

Table 1 Weather conditions at CHES, Chettalli, in the year 2013

Year	Temperature		Relative humidity (%)		Rainfall
	Max.	Min.	Max.	Min.	(mm)
January	31.6	12.3	99.4	24.6	31.7
February	31.5	14.7	98.0	29.8	31.6
March	32.2	16.9	97.7	32.1	32.3
April	34.45	17.98	98.03	17.98	50.8
May	31.36	20.09	97.62	49.81	79.7
June	24.75	19.08	98.81	76.58	308.1
July	24.50	18.84	99.18	80.72	636.3
August	24.94	18.77	99.71	78.52	208.3
September	26.81	18.31	99.86	73.83	195.9
October	28.85	18.31	99.95	64.24	93.7
November	29.23	16.29	100.00	54.88	35.3
December	28.20	12.51	99.75	43.30	0

Table 2 Weather conditions at CHES, Chettalli, in the year 2014

Year	Temperature		Relative humidity (%)		Rainfall
	Max.	Min.	Max.	Min.	(mm)
January	29.33	12.60	100.00	41.95	0
February	31.09	13.6	99.60	38.15	0
March	33.45	14.66	99.41	27.2	13.3
April	33.68	19.00	99.64	39.55	7.0
May	31.78	17.35	85.42	18.85	7.0
June	27.67	19.92	98.88	70.33	11.0
July	25.20	19.04	99.76	82.04	20.0
August	25.65	18.83	100	75.26	18.0
September	27.12	18.60	99.68	74.40	12.0
October	28.62	19.52	100.00	66.81	10.0
November	28.29	15.43	99.00	49.10	1.0
December	27.96	15.64	96.48	50.28	3.0

The fruit pulp was separated from the seeds and blended for a short duration in a homogenizer. The phenol content in karonda pulp was estimated by the Folin-Ciocalteau method at 650 nm, and expressed as gallic acid equivalents (Singleton *et al.* 1999). The flavonoids content was estimated at 510 nm, and expressed in units of catechin equivalents Zhishen *et al.* (1999). Anthocyanin content was estimated at 540 nm, by the method of Di Stefano *et al.* (1989) and expressed as cyanidine equivalents. The antioxidant potential of the pulp of karonda was quantified using DPPH radical scavenging activity (Braca *et al.* 2001) and FRAP (Benzie and Strain 1996) methods, where absorption was read at 517 nm and

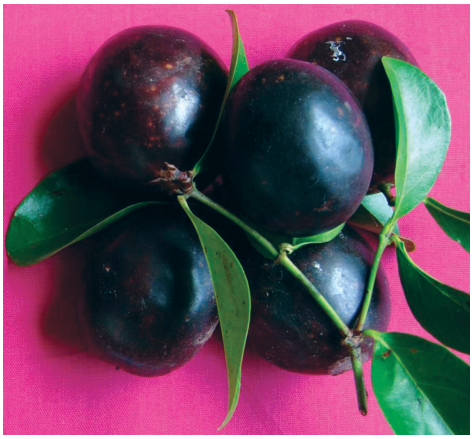

Fig 1a Konkan Bold

Fig 1b CHES K-V/8

Fig 1c CHES K-II-7

Table 3 Morphological characteristics of Konkan Bold, CHES K-II-7 and CHES K-V/8

Characters	Konkan Bold	CHES K-II-7	CHES K-V-8
Fruit weight (g)	18.77	12.14	13.87
Pulp quality	Thick skin, firm, Juicy and sweet, Table purposes	Thin skin, firm, Juicy and sweet Table and processing purposes	Thick skin, firm, Juicy and sweet, Table purposes
Pulp color	Dark blackish red	Dark blackish red	Dark blackish red
Pulp content (%)	83.2	91.03	89.04
TSS (°Brix)	12.80	15.61	16.10
Acidity (%)	2.22	1.07	1.18
Yield (kg/ tree)	30.87	21.80	15.27

593 nm respectively. All observations were recorded in a T80+ UV-Vis spectrophotometer (PG Instruments Corporation, UK).

Profile of phenolic acids and flavonoids:

Chemicals and reagents: Phenolic acid standards-ferulic acid, 2,4-dihydroxybenzoic acid, caffeic acid, gallic acid, gentisic acid, *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxy benzoic acid, protocatechuic acid, salicylic acid, syringic acid, *t*-cinnamic acid, vanillic acid, chlorogenic acid; and flavonoid standards-catechin, hesperetin, apigenin, *naringenin*, myricetin, rutin, luteolin, quercetin, umbelliferone were procured from Sigma Chemical Co., USA. The organic solvents used for the analysis were of chromatographic/MS grade and all other reagents of analytical grade. Water purified in Milli-Q (Millipore) system was used to prepare the mobile phases. All mobile phases were filtered through 0.45 µm pore size membranes. The standard curve for individual phenolic acids and flavonoids were made using different concentration of individual compounds which was identified and quantified by their molecular weight (parent mass m/z) and most abundant fragmented daughters.

Sample preparation and extraction: Total phenols and flavonoids were extracted from 2 g of sample homogenised in 10 ml of 99:1 methanol:HCl. The filtrate was evaporated to dryness under vacuum at 45°C in a rotary flash evaporator, and the residue was dissolved in 3 ml of the mobile phase-0.2% formic acid in methanol, centrifuged, filtered through 0.2 µm nylon filter prior to injection in LC/MS-MS (M/S Waters, Acquity UPLC-H class coupled with TQD-MS/MS with ESI source, from USA), equipped with a degasser, quaternary pump, automatic injection system, with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths and MRM for individual masses. The overall system was controlled by the MassLynx software, which administered data collection and treatment system. The mass spectra obtained using negative ionization mode (ESI^-) for the most abundant forms of deprotonated $[M-H]^-$ molecules of phenolic acids and flavonoids was found by direct sample infusion. These deprotonated molecules were respectively confirmed as precursor ions of the corresponding phenolic acids and flavonoids for the following collision induced decomposition (CID) fragmentation by their respective collision energy (CE) to develop the MRM methods (Tables 4 and 5).

LC and MS-MS conditions: The mobile phase consisted of aqueous phase of 0.1% formic acid in water (A) and organic phase of 0.2% formic acid in methanol (B). The gradient conditions are detailed in Table 6.

The flow rate was 0.3 mL/min. The analytical column used was 2.1 × 50 mm UPLC BEH C_{18} column (Waters, USA) with 1.7 µm particles, protected by a Vanguard BEH C_{18} with 1.7 µm guard column (Waters, USA), and the column temperature was maintained at 25°C. The sample injection volume was 5 µl each time for both phenolic acids and flavonoids. The metabolites eluted were monitored using the UPLC column effluent which was pumped directly without any split into the TQD-MS/MS (Waters, USA) system, optimized for the phenolic acids and flavonoids analysis with source temperature 135 °C, desolvation gas flow of 650 L/hr and temperature at 350 °C.

Data analysis: Quantification of phenols, flavonoids,

Table 4 MRM details of phenolic acids

Compound	Formula/Mass	Parent m/z	Cone volt	Daughters	Collision energy	Ion mode
Caffeic acid	180	178.90	30	135.05	16	ES-
2,4-Dihydroxybenzoic acid	154	152.90	28	65.02	18	ES-
Chlorogenic acid	354	352.97	22	191.10	18	ES-
Ferulic acid	194	192.90	26	134.02	14	ES-
Gallic acid	170	168.90	28	125.03	12	ES-
Gentisic acid	154	152.90	24	108.98	12	ES-
o-Coumaric acid	164	162.90	22	119.06	12	ES-
p-Coumaric acid	164	162.90	24	119.05	14	ES-
p-Hydroxybenzoic acid	138	136.90	26	93.01	12	ES-
Protocatechuic acid	154	152.90	26	109.05	16	ES-
Salicylic acid	138	136.90	28	93.10	14	ES-
Syringic acid	198	196.97	26	182.07	10	ES-
trans-Cinnamic acid	148	146.90	26	103.05	10	ES-
Vanillic acid	168	166.97	26	108.01	20	ES-

Table 5 MRM details of flavonoids

Compound	Formula/Mass	Parent m/z	Cone voltage	Daughters	Collision energy	Ion mode
Apigenin	270	268.97	46	107.04	30	ES-
Catechin	290	289.03	38	245.15	12	ES-
Hesperetin	302	300.97	42	286.15	16	ES-
Kaempferol	286	284.97	54	145.50	36	ES-
Luteolin	286	284.97	54	150.99	26	ES-
Myricetin	318	317.03	42	151.06	28	ES-
Naringenin	272	271.03	34	151.00	16	ES-
Quercetin	302	301.03	36	151.12	20	ES-
Rutin	610	609.10	60	300.20	42	ES-
Umbelliferone	162.14	161.04	42	133.07	18	ES-

anthocyanins and antioxidant potential and characterization of profile of phenols, flavonoids and anthocyanins were performed in triplicates, and mean values with standard error mean are reported. The biochemical parameters were statistically analyzed using *t*-test.

RESULTS AND DISCUSSION

Karonda fruits are rich in the secondary metabolites, phenols, flavonoids and anthocyanins and therefore are a potentially good source of antioxidants. The values of all parameters were higher in the fruits analyzed in 2013 compared to that of 2014, but the trend was identical. The increase in secondary metabolites content and antioxidant activity in karonda fruits analyzed in the year 2013 ranged from 1.5 to 3 times than those analyzed in 2014, with the exception of the flavonoids content in Karonda Bold and the anthocyanins content in all three collections, being at par in both years. This is attributed to the significantly lower rainfall received in 2014 compared to 2013 starting from the flowering stage of March-April to the fruit harvest at June-August, as a result of which the minimum and maximum temperatures were higher, and relative humidity lower in 2014.

The average values of the two year studies indicated that among the three collections studied, the total phenols and flavonoids content were significantly higher in CHES K-V/8, followed by CHES K-II/7 and far less in Konkan Bold. CHES K-V/8 contained 2.5 times higher contents of phenols and flavonoids than the variety Konkan Bold; CHES K-II/7 had 1.6 times greater phenols and flavonoids than Konkan Bold and 2/3rds lesser phenols and flavonoids than CHES K-V/8. The anthocyanin content was at par in the three collections. The antioxidant potential was consequently higher in CHES K-V/8: DPPH radical scavenging activities was 2.3 times and FRAP was 2.4 times higher in CHES K-V/8 compared to Konkan Bold. CHES K-II/7 had 1.7 times greater DPPH radical scavenging activity and 1.6 times higher FRAP activity than Konkan Bold and 1/4th lesser

Table 6 The solvent gradient for separation of phenols and flavonoids.

Time (min)	Solvent A (%) (0.1% formic acid in water)	Solvent B (%) (0.2% formic acid in methanol)	Hold (min)
0	90	10	2.5
4.0	70	30	1.0
5.0	60	40	5.0
10.0	80	20	2.0
14.0	90	10	2.0

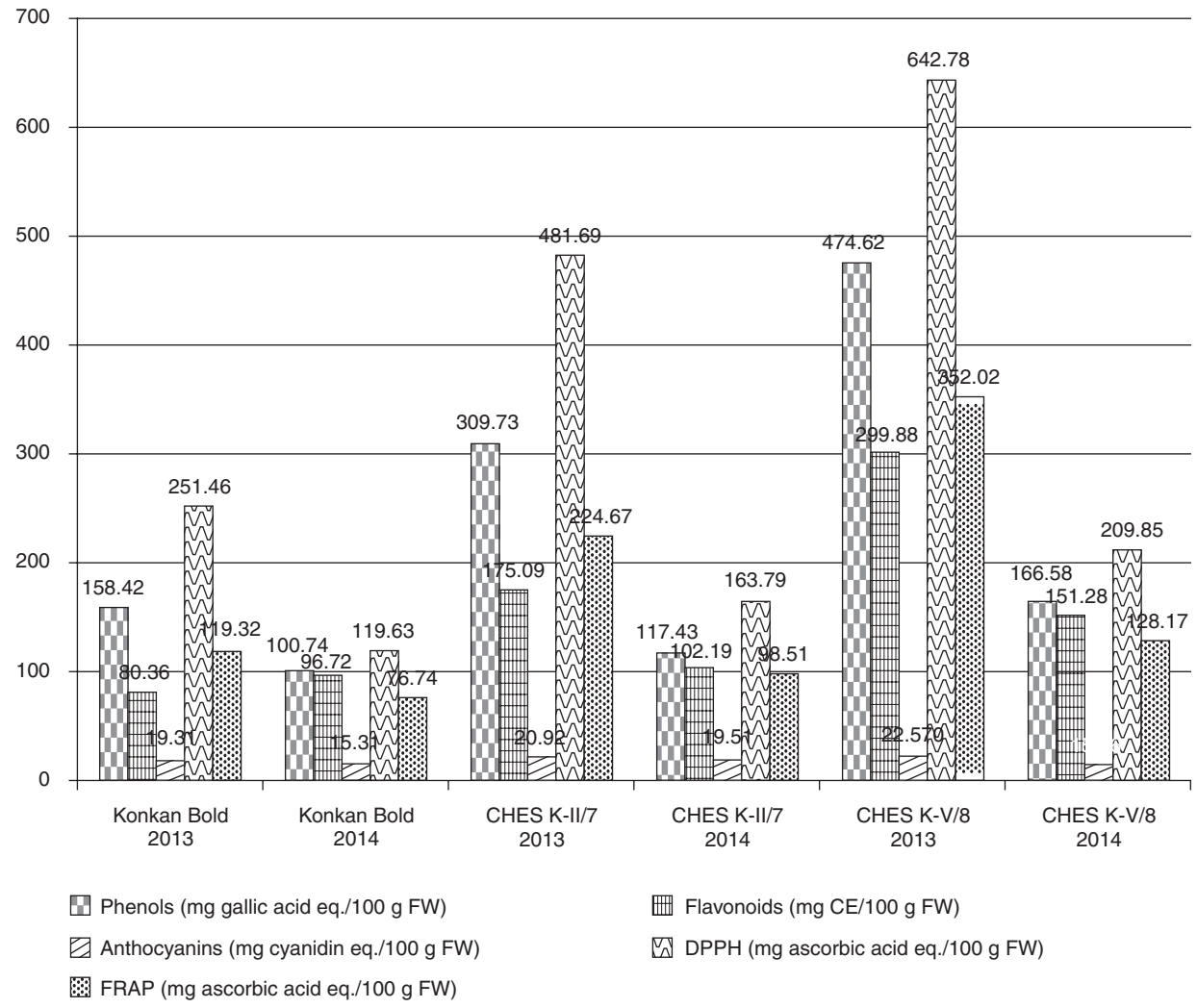

Fig 2 Content of phenol, flavonoids, anthocyanins, and antioxidant potential (DPPH, FRAP activities) in the years 2013 and 2014, in Konkan Bold, CHES K-II-7 and CHES K-V/8.

antioxidant activity than CHES K-V/8 (Fig 2).

Other tropical fruits, more commonly available and widely consumed such as blackberry, carambola, hog plum, honeydew melon, jackfruit, palmyra fruit, pineapple, sapota and wax apple (Mamun *et al.* 2012), contain less phenolic content and possess inferior antioxidant activity compared to the values of karonda fruits reported in this study. Blueberries (*Rubus fructicosus*) are reported to be among the richest sources of natural antioxidants among fruits, with high contents total phenolics (319.3 mg/100 g fwt), total anthocyanins (131.2 mg/100 g fw), and antioxidant activity (ORAC: 46.14 µmol of Trolox/ g fw); in strawberries the total phenols ranged between 123 to 260 mg/100 g fwt. Higher antioxidant activity in blueberries is attributed to the higher levels of anthocyanidins and proanthocyanidins compared to blackberries and strawberries (Huang *et al.* 2012). The total phenols and flavonoids in the karonda samples in the present study compared favourably with strawberries, though the phenols were only half as much and the anthocyanins were only a tenth of blackberries; the anthocyanin content in the karonda samples were twice that of red currants (*Ribes sativum*) (7.7 mg/100 g fw.), less than half of raspberries (*Rubus ideaus*) (40 mg/100 g fw.), and a tenth of blackberries (140 mg/100 g fw.) as reported by Pantelidis *et al.* (2007). However, the total phenol contents reported in these berries were over ten times higher than karonda. The phenol and flavonoid contents were positively correlated with each other and the antioxidant activity (Table 7).

Table 7 Correlation between the total phenols, flavonoids and anthocyanins content to antioxidant potential

	Phenols	Flavo-noids	Antho-cyanins	DPPH	FRAP
Phenols	1.000				
Flavonoids	0.996**	1.000			
Anthocyanins	0.458	0.382	1.000		
DPPH	0.989**	0.973**	0.586	1.000	
FRAP	1.000**	0.996**	0.466	0.990**	1.000

A highly significant correlation was observed between phenol content and DPPH radical scavenging capacity

(0.989), phenol content and FRAP (1.0), flavonoid content and DPPH radical scavenging capacity (0.973), flavonoid content and FRAP (0.996), and expectedly between phenol and flavonoids content (0.996). Lesser correlation was observed between anthocyanin content to other parameters, notably antioxidant activity. Though counter intuitive, similar observations have been made by Orak (2007). Cordenunsi *et al*. (2005) also found no difference in the antioxidant activity between strawberry cultivars despite differences in anthocyanin content and its increase during storage. Whether storage temperature and oxygenation which are reported by Kalt *et al*. (2000) to affect anthocyanin content and antioxidant activity, has played a role here needs to be explored. Thereby, the widely accepted antioxidant function of anthocyanins in specific plants is still a matter of debate. Since polyphenols constitute the majority of secondary plant metabolites and also of dietary antioxidants, the best way to select future cultivars may be to test for high total phenolic content, because this is highly correlated with antioxidant capacity, as seen in the present study. The structural diversity of polyphenols makes the estimation of their content in food difficult (Amiot *et al.* 1992, Hammerstone *et al.* 2000).

Karonda berries exhibited good antioxidant capacity because they possess an array of phenolic compounds. Each plant species has a characteristic signature of phenolic molecules, which may be unique for organs/tissues of that plant. As per the suggestion of Scalbert and Williamson (2000) that it would be desirable to know the nature of the prominent phenolic compounds, the profile of phenols, flavonoids and anthocyanins were determined in Konkan Bold and CHES K-V/8, the collections with highest and lowest phenol contents (Table 8).

The predominant phenols in Konkan Bold and CHES K-V/8 were vanillic acid, followed by protocatechuic acid, *t*-cinnamic acid and ferulic acid; chlorogenic, 2,4-dihydroxybenzoic, syringic, salicylic, caffeic and gentisic acids were present in moderate quantities; *p*-hydroxybenzoic acid, *p*-coumaric acid, gallic acid and *o*-coumaric acid were in negligible amounts. The trend in the chemoprofile of phenolics in CHES K-V/8 was similar to Konkan Bold, except that they were present in significantly larger quantities; varying from over 8.4 times more ferulic acid to 1.2 times more syringic acid, compared to Konkan Bold. Further, ferulic acid, salicylic acid, caffeic acid and gallic acid were also present in higher amounts in CHES K-V/8 compared to Konkan Bold.

Table 8 Profile of phenolic acids in the karonda collections Konkan Bold and CHES K-V/8.

Phenols (µg/gm FW.)	Konkan Bold	CHES K-V/8
Vanillic acid	19747.18	51029.45
Protocatechuic acid	3654.65	5770.16
t-Cinnamic acid	976.50	4612.80
Ferulic acid	835.22	7048.63
Chlorogenic acid	498.52	3493.69
2,4-Dihydroxybenzoic acid	492.17	830.74
Syringic acid	385.15	463.49
Salicylic acid	311.17	2318.62
Caffeic acid	144.48	727.86
Gentisic acid	100.34	319.90
p-hydroxybenzoic acid	51.75	142.14
p-Coumaric acid	37.23	68.19
Gallic acid	31.76	99.18
o-Coumaric acid	1.76	5.29

As in most other plants, the hydroxycinnamic acids-ferulic, caffeic, and *p*-coumaric acids were more common than the hydroxybenzoic acids. The antioxidant capacity of phenolic acids is reported to decrease in the order: protocatechuic acid >caffeic acid >*p*-hydroxybenzoic acid >ferulic acid >vanillic acid >*p*-coumaric acid (Rice-Evans *et al.* 1996, Li *et al.* 2009). The predominant phenols reported in karonda in this study are reported to have specific uses in food and medicine, as for vanillic acid (FAO/WHO Expert Committee on Food Additives, JECFA no. 959; Sinha *et al.* 2008), protocatechuic acid (Kakkar and Bais 2014), *t*-cinnamic acid and its derivatives including chlorogenic acids and salicylic acid (Sharma 2011, Farah *et al.* 2008).

The predominant flavonoids in karonda pulp were rutin, followed by myricetin and umbelliferone; minor quantities of catechin, quercetin, hesperetin, naringenin, luteolin and negligible traces of apigenin (Table 9).

Here too, flavonoids were higher in CHES K-V/8: ranging from 25 times more luteolin to 9% higher catechin compared to Konkan Bold; myricetin was the only flavonoid 53% lesser in CHES K-V/8 compared to Konkan Bold. The glucosides of cyanidin (cyanidin-3-rutinoside, cyanidin-3-O-acetyl glucoside and cyanidin-3-O-monoglucoside) and pelargonidin (pelargonidin-3-(acyl)-diglucoside-5-glucoside and pelargonidin-3-(feruloyl)-glucoside) were the anthocyanins detected.

Rutin, also found in black tea, buckwheat bran, many citrus fruits and apple skins, helps the body utilize vitamin C, umbelliferone has antioxidant properties and absorbs UV light strongly (Lupei *2008*). Anthocyanins are responsible for the colour in fruits, and have potent antioxidant/ antiinflammatory activities (Pandey and Rizvi 2009).

Singh and Uppal (2015) reported that crude karonda root extracts contain small quantities of alkaloids, flavonoids, saponins and large amounts of cardiac glycosides,

Table 9 Profile of flavonoids in the karonda collections Konkan Bold and CHES K-V/8.

Flavonoids (µg/gm fw)	Konkan Bold	CHES K-V/8
Rutin	8296.07	19749.95
Myricetin	2473.95	1167.18
Umbelliferone	255.32	1516.23
Catechin	104.27	113.75
Quercetin	61.57	306.59
Hesperetin	22.53	52.17
Naringenin	30.03	109.65
Luteolin	16.51	406.61
Apigenin	1.98	2.47

triterpenoids, phenolic compounds and tannins. They also contain volatile principles 2-acetyl phenol, lignan, carinol, sesquiterpenes (carissone, carindone), lupeol, β-sitosterol, 16-β-hydroxybetulinic acid, β-amyrin and β-sitosterol glycoside, and des-N-methylnoracronycine, an acridone alkaloid. Karonda stem is reported to contain sesquiterpene glucosides, leaves contain triterpenoid and tannins, and a new isomer of urosolic acid namely carissic acid triterpene carandinol, betulinic acid, β-sitosterol-3-O-β-d-glucopyranoside, oleanolic acid, ursolic acid, and 4-hydroxybenzoic acid. Karonda fruits have been reported to contain carisol, epimer of β-amyrin, linalool, β-caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol and β-sitosterol.

The mechanisms by which phenolic compounds act as protective antioxidants are by H atom transfer (gallic acid, caffeic acid, epicatechin, quercetin), single electron transfer and metal chelation (quercetin). The flavonol myricetin is also a good antioxidant; and myricetin, catechin and quercetin were present in significant quantities in the karonda collections (Table 8). The anthocyanins glucosides of cyanidine and pelargonidin in karonda are equipotent to quercetin and catechin gallates, provided that a catechol structure is present in ring B (Rice-Evans *et al.* 1996). The benefits of dietary polyphenols and flavonoids are limited by their low bioavailability; even on a polyphenol rich diet, the plasma concentration does not exceed 1-7 mmol/L *in vivo*, due to extensive metabolism and regulatory mechanisms intended to prevent toxicity of flavonoids. In plants, phenolics are widely distributed in conjugated forms of esters and amides, but rarely as glycosides and seldom as the free acids that cannot be absorbed in native form (Robbins 2003). Generally, aglycones can be absorbed from the small intestine.

CONCLUSION

The importance of plant phenols, flavonoids and anthocyanins for their antioxidant potential has been borne out by the enormous literature supporting the claim. There is no data till date in karonda, which has future potential for table purpose as well as processed value-added products, with the additional benefit of being therapeutic. It is evident from the present study that karonda is a potential source of health promoting polyphenols, found in significant amounts when compared to other more commonly consumed berries or under-utilized fruits. The phytochemical profile of phenolic acids revealed the predominance of protocatechuic, *t*-cinnamic, ferulic, chlorogenic, 2,4-dihydroxy benzoic, syringic and salicylic acids; the flavonoids rutin, myricetin and umbelliferone; and the anthocyanins cyanidine and pelargonidin glucosides. Studies on the bioavailability of these polyphenols would reveal exactly how useful these phenols are to human health. Furthermore, the collections CHES K-V/8, followed by CHES K-II/7, in addition to having favourable horticultural traits, were found superior to the variety Konkan Bold in its polyphenol content and antioxidant activity, and therefore may be recommended for release as a variety. The results indicate a potential market role for karonda as a functional food ingredient or nutraceutical. These results make a strong case in favour of the conservation and breeding for superior varieties of karonda, at present a vastly neglected plant.

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