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Physico-chemical characteristics and antioxidant potential of selected underutilized fruits from North Gujarat

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Abstract
Five underutilized fruits from North Gujarat region viz. *Karonda*, *bael*, *wood apple*, *jamun* and *fig* were evaluated for their TS, TSS, titratable acidity, pH, ascorbic acid. Hydrophilic and lipophilic extracts were prepared from all the fruit pulp and analyzed for antioxidant activity and TPC content. The bael and karonda pulp showed higher TS and TSS content as compared to other fruits. The total phenolic and vitamin C content of bael pulp was highest as 14.16 µmol GAE/g and 65.32 mg/100g, respectively, while, karonda pulp also showed comparatively higher value of total phenolic content (7.88 µmol GAE/g) and vitamin C content (24.56 mg/100g) as compared to wood apple, jamun and fig. The antioxidant activity of hydrophilic and lipophilic extracts measured by ABTS and DPPH method was significantly ($p<0.05$) higher for bael and karonda. Further, the hydrophilic extract showed significantly ($p<0.05$) higher antioxidant activity as compared to lipophilic extract. Antioxidant activity showed a very high positive correlation with the TPC and Vitamin C content. Hence, it may be concluded that bael and karonda could be potent source of antioxidant components for the development of value-added dairy and food products.

Keywords: Underutilized fruit, hydrophilic, lipophilic, antioxidant, total phenolic content, total solids, total soluble solids

Introduction

Fruits and vegetables contain various bioactive compounds and a number of these naturally occurring substances have been recognized to have antioxidant potential. Flavonoids and phenolic compounds are among the major contributors to antioxidant activity. These compounds may work independently or synergistically (Kang *et al*., 2003) [14]. There are many fruits available in India such as *bael*, *karonda*, *Jamun*, *wood apple* and *fig* which are highly nutritious and cheaply available but have not been fully exploited in the value addition of dairy and food products. Exploitation of these underutilized fruits in the development of value added dairy and food products has been recently gaining considerable interest because of their high nutritional value and health benefits (Singh and Nath *et al.*, 2004; Vino, 2016; Jadhao *et al.*, 2018) [29, 34, 12].

Bael *(Aegle marmelos)* contains various vitamins and minerals which include carotene, thiamine, riboflavin, niacin & vitamin C and calcium, phosphorus & iron. It also contains a large number of coumarins, alkaloids, sterols and essential oils and found to have analgesic, anti-inflammatory, antipyretic, anti-microfilaria, antifungal, hypoglycemic, antidyslipidemic, immunomodulatory, antiproliferative, wound healing, anti-fertility and insecticidal properties (Neeraj *et al*., 2017) [17]. This fruit used as ayurvedic remedy against diarrhea, dryness of the eye and common cold. It is also useful in preventing scurvy and stomach ailments (Dnyaneshwar *et al*., 2006) [8].

Karonda (*Carissa carandas*) is a fruit which tastes sour and astringent & are rich in iron and contains a good amount of protein, carbohydrates, fat, fibre, vitamin C and calcium. The fruits are traditionally used for medicinal treatments of malaria, epilepsy, nerve disorder, headache, fever itches & leprosy and acts as blood purifier & pain reliever. The major bioactive constituents include alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins (Reshu, 2017) [25].

Jamun (*Syzygium cumini*) is a very nutritious summer fruit that has a wide range of therapeutic properties such as liver stimulation, digestive, carminative, coolant and shows hypoglycaemic effect (Kapoor and Renote, 2016) [15]. It has been recognized as a neutraceutical due to presence of phytonutrients such as anthocyanins, tannins, alkaloids, terpenoids and various minerals (Chaudhary and Mukhopadhyay, 2012) [7].

Wood apple *(Feronia limonia)*, which is similar to bael fruit, is a rich source of calcium, phosphorus, iron and vitamins like carotene, riboflavin, niacin, thiamine and vitamin C. It is used as a liver and cardiac tonic, and when unripe, for easing diarrhoea and dysentery (Intekhab and Aslam, 2009) [11]. Figs *(Ficus carica)* are consumed as fresh, dried, preserved, candied or canned. It contains protein, calcium, iron, vitamin A and thiamine at varying concentration (Aljane and Ferchichi, 2009) [3].

In-spite of these underutilised fruits are cheap and have nutritional and medicinal values, they are not fully exploited in dairy and food products for value addition. Several studies have been carried out on the physico-chemical properties of underutilised fruits (Shaheel *et al.*, 2015; Azeez *et al.*, 2016; Umeshagouda *et al.*, 2017; Sawale *et al.*, 2018; Akhila and Umadevi, 2018) [27, 5, 33, 26, 2]. However, only few studies have been reported on their antioxidant activity (Rajitha, 2013; Pellagrani *et al.*, 2003) [22, 19]. Therefore, there is a need to study the antioxidant potential of these underutilised fruits to explore the possibility of their utilization in the development of value added dairy and food products with enhanced antioxidant activity, intended to be used in the prevention of diseases caused by oxidative stress. In this study the physico-chemical characteristics and antioxidant potential of some of the underutilised fruits have been evaluated.

Materials and Methods

Raw materials and Chemicals

Fresh and ripened *karonda*, *bael*, *wood apple*, *jamun* and *fig* fruits were procured from Horticulture farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. All the chemicals used in the research work were of analytical grade (AR) and HPLC grade.

Preparation of fruit pulp and extract

Fruits were thoroughly cleaned with potable water to remove adhering dirt and seeds were removed manually using knife and the pulp was obtained by smashing the fruit using food smasher. The pulp was packed in 150 ml sterile plastic container and stored at -18°C till further use. Hydrophilic and lipophilic extracts of the fruit pulps were prepared according to the method described by Nilsson *et al*. (2005) [18] and were analysed for their antioxidant activities and TPC contents.

Physico-chemical analysis of fruit pulp

All the fruit pulps were analysed for total solids (TS), total soluble solids (TSS), pH, titratable acidity (TA) and ascorbic acid by the method of Ranganna (2012) [23].

Antioxidant activity and Total phenolic content (TPC) of fruit pulps extracts

Hydrophilic & lipophilic extracts of fruit pulps were analysed for antioxidant activity by ABTS radical scavenging assay as described by Re *et al.* (1999) [24] and DPPH radical scavenging assay as described by Brand Williams *et al.* (1995) [6] with slight modification. The extracts were also analysed for TPC by the method of Kahkonen *et al.* (1999) [13].

Statistical analysis

The data related to physico-chemical characteristics and antioxidant activity of the samples was analysed using statistical design CRD with equal number of observations as per the method of Snedecor and Cochran (1994) [30]. The bivariate correlation between total phenolic content, Vitamin C and antioxidant activity measured using different methods were analysed.

Results and Discussion

Chemical characteristics of fruit pulps

Fruit pulp of five selected underutilized fruits viz., Karonda (*Carissa carandas* L.), Bael (*Aegle marmelos* L.), Wood apple (*Limonia acidissima* L.), Jamun (*Syzygium cumini* L.) and Fig (*Ficus carica* L.) were prepared and analyzed for their physico-chemical characteristics viz., TS, total soluble solids (TSS), titratable acidity (TA), pH and ascorbic acid and the results are presented in Table 1.

Table 1: Chemical characteristics of pulp of selected underutilised fruits

Fruit pulp	Total Solids (%)	Total Soluble Solids (^{0}Brix)	pH	Titratable Acidity (as % Citric acid)
Karonda	16.12±0.42	15.2±0.2	4.28±0.02	0.48±0.02
Bael	40.26±0.58	38.4±0.3	4.82±0.03	0.32±0.04
Wood apple	28.33±0.21	26.6±0.1	3.76±0.01	0.83±0.05
Jamun	18.24±0.15	17.4±0.2	4.20±0.02	0.44±0.03
Fig	19.67±0.17	18.5±0.1	4.60±0.03	0.14±0.02
Data are presented as means±SD (n=3)				

The TS and TSS content of fruit pulps obtained from *karonda*, *bael*, *wood apple*, *jamun* and *fig* were 16.12, 40.26, 28.33, 18.24 & 19.67% and 15.2, 38.4, 26.6, 17.4 & 18.5°Brix, respectively. Among all the fruits studied, the bael pulp showed the highest TS and TSS content of 40.26% and 38.6°Brix while karonda showed the lowest values of 16.12% and 15.2° Brix, respectively.

The pH of different fruit pulps varied from 3.76 to 4.82 with the highest value of 4.82 for bael and lowest value of 3.76 for wood apple. The acidity ranged from 0.14 to 0.83% as citric acid with the highest value of 0.83% as citric acid for wood apple and lowest value of 0.14% as citric acid for fig.

The TSS content of the karonda pulp obtained in the present investigation is in the close proximity with the studies carried out by Azeez *et al*. (2016) [5]. The titratable acidity in terms of citric acid and pH of the pulp obtained were similar to the results reported by Shirish *et al.* (2014) [28]. These results obtained for bael pulp were also comparable with the results of Tarsem and Gehlot (2006) [32] who reported mean values of TSS (°Brix), TA (%) and pH as 36.3, 0.46 and 4.5, respectively. Other workers (Karnale, 2017 and Sawale *et al.*, 2018) [16, 26] also reported similar values for TA (%) and pH of bael pulp. For wood apple, similar results were obtained by Ranjitha (2013) [22] who reported 36.80 ºBrix, 3.77 and 0.91

as TSS content, pH and titrable acidity in terms of citric acid. The values of TSS content, pH and titrable acidity for Jamun fruit pulp reported in the study were within the range of values reported by Ghose *et al*. (2017) [9], Akhila and Umadevi (2018) [2] and Jadhao *et al*. (2018) [12]. Abbas *et al.* (2016) [1] reported TSS content, pH and titrable acidity as 11.54° Brix, 4.8 and 0.08%, respectively for fig pulp. It was revealed from the results that the chemical characteristics of different fruit pulp varied with the type of fruit.

Antioxidant potential of selected fruit pulps

All the five fruit (*karonda, bael, wood apple, jamun* and *fig*) preparations were evaluated for their antioxidant potential. The total antioxidant capacity is contributed by a mixture of different bioactive components, which may exhibit different mechanisms of action with synergistic and/or antagonistic interactions. Therefore, it is necessary to use more than one method to determine *in-vitro* antioxidant capacity of fruits. The hydrophilic and lipophilic extracts of fruits were prepared and antioxidant activity was measured following two most commonly used methods based on ABTS and DPPH radical scavenging activity. The total antioxidant capacity was determined by the sum of hydrophilic and lipophilic antioxidant values obtained by each of the method. The fruit pulps were also analyzed for their total phenolic and vitamin C contents.

Antioxidant potential and TPC contents of hydrophilic extract of fruit pulps

The antioxidant capacities of hydrophilic extracts of selected fruits and their total phenolic contents are shown in Table 2. The ABTS radical scavenging activity of hydrophilic extracts for *karonda, bael, wood apple, jamun* and *fig* were found as 4.78, 8.65, 1.68, 3.80, and 2.62 µmol TE/g, respectively. As compared to other fruits, the bael pulp extracts exhibited highest ABTS radical scavenging activity of 8.65 µmol TE/g. It was observed from the results that the ABTS radical scavenging activity of hydrophilic extracts from different fruit pulp varied significantly (p<0.05) between the fruits. The DPPH radical scavenging activities of hydrophilic extracts of fruit pulps varied from 1.29 to 5.32 µmol TE/g. Again, the bael exhibited highest value of 5.32 µmol TE/g, whereas, the fig exhibited lowest values of 1.29 µmol TE/g for DPPH radical scavenging activity. Significant (p<0.05) differences were observed between the DPPH radical scavenging activity of the hydrophilic extracts of all the fruit pulps except *wood apple* and *fig*. Similar trend was also observed for total phenolic content. The total phenolic content of hydrophilic extracts of fruit pulps varied from 2.89 to 10.32 µmol GAE/g. Similar to the antioxidant activity, the bael pulp was found to have highest total phenolic content (10.32 µmol GAE/g) among all the fruits studied.

Table 2: Antioxidant potential and TPC contents of hydrophilic extract of fruit pulps

Fruit Pulp	Antioxidant capacity (µmol TE*/g)		TPC (µmol GAE**/g)
	ABTS	DPPH	
Karonda	4.78±0.04^{b}	3.44±0.06^{b}	5.56±0.14^{b}
Bael	8.65±0.03^{a}	5.32±0.02^{a}	10.32±0.15^{a}
Wood apple	1.68±0.05^{e}	1.64±0.04^{d}	2.89±0.74^{d}
Jamun	3.80±0.07^{c}	2.38±0.05^{c}	4.66±0.13^{c}
Fig	2.62±0.02^{d}	1.29±0.07^{d}	3.22±0.15^{d}

Means with different superscripts in each column (a, b, c, d, e) were significantly (p<0.05) different from each other. Data are presented as means±SD (n=4)
*-TE- Trolox Equivalent **-GAE- Gallic Acid Equivalent

Antioxidant potential and TPC contents of lipophilic extracts of fruit pulp

The antioxidant capacities of lipophilic extracts of selected fruits and their total phenolic contents are shown in Table 3. The ABTS and DPPH radical scavenging activity of lipophilic extract varied significantly (p<0.05) between the fruits ranging from 0.85 to 2.98 µmol TE/g and 0.53 to 1.96 µmol TE/g except for wood apple and fig. Total phenolic content of the bael pulp was found maximum (3.84 µmol GAE/g) followed by *karonda*/*jamun* and *wood apple/fig* (Table 3). The bael pulp lipophilic extracts showed highest ABTS and DPPH radical scavenging activity as well as total phenolic content, while wood apple and fig showed the lowest values.

Table 3: Antioxidant potential and TPC contents of lipophilic extracts of fruit pulp

Fruit Pulp	Antioxidant capacity (µmol *TE/g)		TPC (µmol GAE**/g)
	ABTS	DPPH	
Karonda	1.58±0.03^{b}	1.22±0.02^{b}	2.32±0.10^{b}
Bael	2.98±0.01^{a}	1.96±0.04^{a}	3.84±0.15^{a}
Wood apple	0.86±0.02^{d}	0.53±0.03^{d}	1.32±0.13^{c}
Jamun	1.27±0.04^{c}	0.82±0.02^{c}	2.25±0.19^{b}
Fig	0.85±0.03^{d}	0.55±0.03^{d}	1.22±0.14^{c}

Means with different superscripts in each column (a, b, c, d) were significantly (p<0.05) different from each other. Data are presented as means±SD (n=4)
*-TE- Trolox Equivalent **-GAE- Gallic Acid Equivalent

From the study of antioxidant activity by ABTS and DPPH radical scavenging assay and TPC content of both hydrophilic and lipophilic extract, it was observed that all the values were higher for hydrophilic extract as compared to lipophilic extract. Arpit (2008) [4] also reported higher antioxidant activity for aqueous extract of strawberry using ABTS and DPPH radical scavenging assay (15.13 and 14.29 µmol TE/g) and high total phenolic content (16.18 µmol GAE/g) as compared to lipophilic extract with corresponding values of 4.68 & 5.73 µmol TE/g and 4.14 µmol GAE/g. Higher antioxidant activity in the aqueous extract was attributed to increased total phenolic content in the extract.

Total antioxidant activity, total TPC and Vitamin C content of fruit pulps

The total antioxidant capacity of fruit pulps was determined by sum of antioxidant values of hydrophilic and lipophilic extract measured by ABTS and DPPH method. The total phenolic content was also determined by sum of phenolic content in both hydrophilic and lipophilic extracts. The vitamin C content of the fruit pulp was measured using N-bromosuccinimide and the results obtained are presented in Table 4.

Table 4: Total antioxidant activity, total TPC and Vitamin C content of fruit pulps

Fruit Pulps	Antioxidant capacity (μmol TE*/g)		TPC (μmol GAE**/g)	Vitamin C (mg/100 g)
	ABTS	DPPH		
Karonda	6.36 ± 0.04^{b}	4.66 ± 0.04^{b}	7.88 ± 0.10^{b}	24.56 ± 0.41^{b}
Bael	11.63 ± 0.02^{a}	7.28 ± 0.03^{a}	14.16 ± 0.14^{a}	65.32 ± 0.72^{a}
Wood apple	2.54 ± 0.03^{e}	2.17 ± 0.04^{e}	4.21 ± 0.13^{d}	9.90 ± 0.39^{d}
Jamun	5.07 ± 0.04^{c}	3.20 ± 0.02^{c}	6.91 ± 0.17^{c}	18.97 ± 0.23^{c}
Fig	3.47 ± 0.02^{d}	2.84 ± 0.01^{d}	4.44 ± 0.12^{d}	8.65 ± 0.18^{d}

Means with different superscripts in each column (a, b, c, d, e) were significantly different (p<0.05) from each other. Data are presented as means±SD (n=4)
*-TE- Trolox Equivalent **-GAE- Gallic Acid Equivalent

It is evident from the results that the ABTS radical scavenging activity of different fruit pulps varied significantly (p<0.05) ranging from 2.54 to 11.63 μmol TE/g. The wood apple and fig showed relatively lower ABTS radical scavenging activity as compared to karonda, bael and jamun. Similar trend was also observed for DPPH radical scavenging activity. It was found that the ABTS values were relatively higher as compared to DPPH values (Table 4). The bael showed highest ABTS value of 11.63 μmol TE/g and wood apple showed lowest value of 2.54 μmol TE/g. In case of DPPH radical scavenging activity, the bael showed highest value of 7.28 μmol TE/g, whereas, the wood pulp showed lowest value of 2.17 μmol TE/g. The TPC and Vitamin C contents varied significantly (p<0.05) from 4.21 to 14.16 μmol GAE/g and from 8.65 to 65.32 mg/100 g, respectively, among all the fruit pulps except wood apple and fig for which no significant difference was observed. Bael pulp showed highest values for both TPC and vitamin C contents as compared to other fruit pulps. Overall, it was evident from the Table 4 that the antioxidant activity of the fruit pulp is directly proportional to the TPC content and vitamin C content. Therefore, the higher total antioxidant activity (ABTS and DPPH) of bael pulp may be attributed to higher total phenolic content (14.16μmol GAE / g) and vitamin C content (65.32 mg / 100g). Hakkinen *et al.* (1999) [10] in his study reported that the higher antioxidant activity of water soluble extract may be due to high amount of ascorbic acid. Szeto *et al.* (2002) [31] observed that increase in the TSS content increased the antioxidant activity of fruit pulp indicating the contribution of soluble components to the antioxidant activity of the fruit pulp. Rajan *et al.* (2011) [21] reported higher concentration of flavonoids (129 mg/g) in aqueous extract of bael pulp. The high antioxidant capacity of karonda fruit pulp could also be ascribed to its high total phenolic content (7.88 μmol GAE /g) and high vitamin C content (24.56 mg/ 100 g). It was reported that karonda contain wide range of antioxidant components such as alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins (Rahmatullah *et al.* 2009) [20]. It is inferred from the results that the higher total antioxidant activity of bael and karonda fruit pulp is attributed to high total phenolic and vitamin C content.

Further, the correlation among ABTS, DPPH, TPC and Vitamin C was also determined and results are presented in the Table 5. A high positive correlation was observed among four parameters describing antioxidant capacity (ABTS and DPPH assay), total phenolic (TPC) and Vitamin C content of fruit pulp. The highest correlation was obtained between ABTS to DPPH values and ABTS to TPC values (r = 0.99). The correlation between DPPH to TPC values was r = 0.98. Vitamin C to ABTS, DPPH and TPC showed correlation of r = 0.98, r = 0.97 and r =0.99, respectively.

Table 5: Correlation Coefficient between ABTS, DPPH, TPC and Vitamins C of fruit pulps

	ABTS	DPPH	TPC	VITAMIN C
ABTS	1			
DPPH	0.99	1		
TPC	0.99	0.98	1	
VITAMIN C	0.98	0.97	0.99	1

Conclusion

The evaluation of physico-chemical characteristics and antioxidant activity of selected underutilized fruits viz., *bael, karonda, Jamun, wood apple* and *fig* revealed that the bael pulp had significantly high concentration of TS, TSS, Vitamin C and TPC content and antioxidant activity as compared to other fruits. The karonda pulp also found to have a good amount of TPC and vitamin C content as well as antioxidant activity compared to jamun, wood apple and fig. A high positive correlation was observed between antioxidant activity and TPC and Vitamin C content of the fruits. The hydrophilic extract of the selected fruits showed significantly higher antioxidant activity as compared to lipophilic extract. The ABTS scavenging activity in both the extract were higher than the DPPH scavenging activity. Thus, it may be concluded that both bael and karonda has potential to be utilized as a source of antioxidant rich ingredient to develop value added dairy and food products.

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