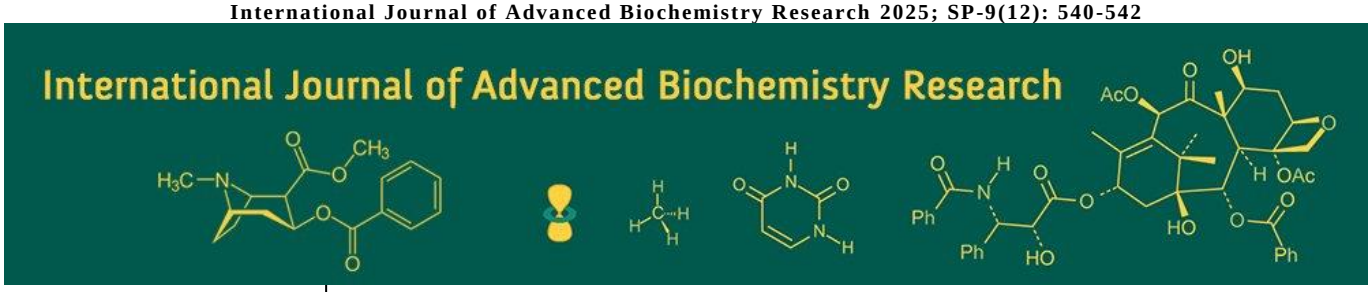

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Investigation of biochemical components and antioxidant activity of karonda squash

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Abstract
Standardization and storage studies of karonda squash were conducted to track changes in its biochemical composition and antioxidant activity over time. After six months of storage the squash retained significant levels of total carotenoids, total flavonoids and total phenols, including antioxidant activity assessed through FRAP assay and DPPH radical scavenging activity.

Keywords: Karonda, bio chemical composition, Squash, antioxidant activity, storage studies, processing, value addition

Introduction
Karonda (*Carissa carandas* L.) a Apocynaceae member, is an indigenous, hardy plant, underutilized in mainstream horticulture. Thriving where other fruit crops struggle, this evergreen spiny shrub is resilient enough to yield satisfactory results even in marginal soil, unlike many other fruit varieties (Tripathi *et al.*, 2014) [13]. Its dense, thorny growth habit has traditionally made it an effective bio-fence or hedge plant for protecting fields and gardens.
While Karonda is gaining popularity due to its rich source of nutrients (notably Vitamin C and Iron) and antioxidants, its fresh consumption is limited. The fruit, particularly when unripe, is distinctly acidic and astringent, making it difficult to be relished in large quantities (Bajpai *et al.*, 2015) [2]. This high acidity, however, makes the fruit an ideal candidate for value addition and processing. Converting the fruit into popular products like squash, jams, jellies, and pickles effectively mitigates its tartness and astringency. The processing helps in creating useful Products. For Socio-Economic Development in Rural India (Srivastava *et al.*, 2017) [11]. Processing also helps in handling large scale production of Karonda fruits when used as a fence and also plays a role in income generation. The development and standardization of Karonda squash, therefore, represent a significant step in promoting the wider use of this resilient and nutritionally valuable underutilized fruit.

Material and Methods
The study investigating the potential of Karonda squash preparation was conducted in 2018. Research activities and analysis were carried out at College of Horticulture, Rajendranagar, Hyderabad, SKLTGHU and the Central Instrumentation Cell (PJTAU). Karonda fruits were sourced from the Fruit Research Station, Sangareddy, SKLTGHU, and all other necessary ingredients were purchased from the local market.

Total Carotenoids
Total carotenoids were determined following the method described by AOAC (2000) [1]. A one-gram (X) sample was first saponified with alcoholic KOH for 20 minutes at 37 °C. The carotenoids were then repeatedly extracted from the saponified mixture into petroleum ether using a separating funnel until the solvent layer became clear. The volume of the pooled petroleum ether was recorded (N ml), and its absorbance (M) was measured at 450 nm.

$$\text{Total carotenoids microgram/g sample} = \frac{4 \times N \times M \times 1000}{X \text{ mg}}$$

Total Phenols

The Folin-Ciocalteu Reagent (FCR) method, as described by Singleton *et al.*, 1999 [10], was used to quantify total phenols. The following procedures are involved in the colorimetric assay: 0.5 ml of diluted FCR (1:1), 10 ml of sodium carbonate, and 0.2 ml of the sample's methanol extract. After making up to 12 ml with distilled water, the mixture is incubated for 60 minutes at 37 degrees Celsius. At 750 nm, the resultant solution was subsequently measured using spectrophotometry (Kamalaja and Prashanthi, PJTAU). A Gallic Acid standard was used to determine the results.

$$\text{Total Phenols (GAE mg/100 g)} = \frac{\text{Sample OD x Standard concentration x Volume made up x 100}}{\text{Sample weight (g) x Standard OD x Aliquot taken x 1000}}$$

Total Flavonoids

A colorimetric assay (Zhishen *et al.*, 1999) [15] was used to measure the total flavonoids expressed as Rutin Equivalents per 100 g. After preparing a measured volume of the sample's methanol extract, it was diluted with distilled water to a final amount of 5 ml. 0.3 milliliters of sodium nitrite were added to this. 0.6 cc of 10% aluminum chloride was added and well mixed after a 5-minute wait. 2 milliliters of 1N sodium hydroxide and 2.1 milliliters of distilled water were added. After then, the mixture was well blended. Using a UV-visible spectrophotometer and a blank for references, the final pink solution's absorbance was measured at 510 nm (Kamalaja and Prashanthi, PJTAU).

$$\text{Total Flavonoids (RE mg/100 g)} = \frac{\text{Sample OD x Standard concentration}}{\text{Sample weight (g) x Standard OD x Aliquot taken x 10}}$$

DPPH Radical Scavenging Activity

The 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging assay (Brand-Williams *et al.* 1995) [3], was used to measure antioxidant activity. This technique is predicated on the antioxidants in the sample reducing the stable, purple-colored DPPH radical, which results in a detectable color shift. A UV spectrophotometer is used to measure the solution of purple color intensity at 517 nm. By comparing the absorption of the resulting oxidized solution to a methanol blank, the reduction of the DPPH radical was ascertained. Trolox Equivalents mg/100 g sample was the measure of the sample's total antioxidant capacity as determined by the DPPH assay (Kamalaja and Prashanthi PJTAU).

$$\text{Percent inhibition} = \frac{\text{AC-AE}}{\text{AE}} \text{ x } 100$$

Where,
AC-Absorption of control
AE-Absorption of extract or standard

$$\text{TAC by DPPH assay TE mg/100 g} = \frac{\text{Volume made up x std. conc. x sample \% inhibition}}{\text{Aliquot taken x sample weight (g) x sample \% inhibition x 10}}$$

Ferric Reducing Antioxidant Power Assay

The Ferric Reducing Antioxidant Power assay, following the methodology outlined by Benzie and Strain (1996) [4] and Tadhani *et al.* (2007) [12], was employed to assess the Ferric Reducing Antioxidant Power of the Karonda squash.The reduction of ferric-2,4,6-tris (2-pyridyl-s-triazine) complex (Fe3+-TPTZ) to its intensely blue ferrous form (Fe2+-TPTZ) at an acidic pH by antioxidants of the sample is the principle of assay. The procedure entailed diluting a specified volume of the methanol extract to 0.3 ml using distilled water, subsequently incorporating 1.8 ml of FRAP reagent. The resulting solution after 10-minute incubation at 37 °C, is measured for absorbance at 593 nm. Ferric Reducing Antioxidant Power of Karonda squash is expressed as Trolox Equivalents mg/100 g (Kamalaja and Prashanthi).

Statistical Analysis

Completely Randomized Design is used to analyze data for interpretation.

Results

Total Carotenoids (mg/100 g)

The changes observed in carotenoid levels throughout the six-month storage duration of karonda squash are shown in Table 1. The peak content was identified in karonda squash immediately after preparation quantified at 0.312 mg/100 g. The overall carotenoid content observed substantially decreased from the second month of storage to the sixth month. The minimum concentration was documented at the end of the six-month duration, at 0.254 mg/100 g. Carotenoids are more prone to auto-oxidation during storage period might be a reason for a fall in carotenoid content. The unsaturated chemical structure makes it more susceptible to thermal breakdown and oxidation.

Similarly Harshita *et al.* (2016) [5] conducted an analysis of mango ready-to-serve drinks and squash, examining alterations in their chemical composition over a three-month storage period at monthly intervals. They reported a decline in total carotenoids in both beverages as the storage duration increased.

Total Phenols (Gallic acid equivalents mg/100 g)

The alterations observed in the total phenolic content during the six-month storage of karonda squash are detailed in Table 1. The highest level was noted in freshly processed karonda squash, measuring 34.96 GAE mg/100 g. A significant decline in total phenol content was recorded starting from the third month of storage, continuing until the sixth month, where it reached 31.54 GAE mg/100 g. The reduction in phenolic content may be attributed to oxidation and polymerization reactions. Comparable findings were documented by Sharma *et al.* (2012) in their study of guava-jamun blended squash. Karpagavalli and Amutha (2015) [8] indicated a gradual decline in total polyphenols in pomegranate squash throughout the storage period. Additionally, Harshita *et al.* (2016) [5] noted a decrease in total phenols in storage of ready-to-serve mango drink and squash.

Total Flavonoids (RE mg/100 g)

The total content of flavonoids of karonda squash throughout the six-month storage is documented in Table 1. The maximum concentration of total flavonoids (128.68 RE mg/100 g) was recorded in karonda squash immediately after preparation, with no significant alterations detected over a two-month storage period. However, a notable decline was recorded in the latter part of the storage period, reaching a minimum of 113.96 RE mg/100 g. Karpagavalli

and Amutha (2015) [8] similarly noted a gradual reduction in total flavonoids in pomegranate squash over a storage period of 180 days.

Ferric Reducing Antioxidant Power Assay (FRAP Assay) (TE mg/100 g)

Ferric Reducing Antioxidant Power capacity was assessed throughout the six-month storage with findings displayed in Table 1. The peak FRAP value (13.56 TE mg/100 g) was observed at the 0 month and exhibited a notable decrease from the second month of storage to the sixth month (6.64 TE mg/100 g). The decline in ascorbic acid, carotenoids, phenols, and flavonoids during the storage might be responsible for decrease in Ferric Reducing Antioxidant Power capacity of karonda squash. Karpagavalli and Amutha (2015) [8] showed a reduction in antioxidant activity during storage.

DPPH (TE mg/100 g)

The DPPH radical scavenging activity expressed as Trolox equivalents, of karonda squash was quantified over a six-month storage period, with findings presented in Table 1. The peak DPPH radical scavenging activity was seen at 0 months (67.85 TE mg/100 g), with a notable decline from the third month to the sixth month of storage (54.20 TE mg/100 g). Diminished levels of ascorbic acid, carotenoids, phenols, and flavonoids might be the reason for decrease in DPPH radical scavenging activity. Karpagavalli and Amutha (2015) [8] showed a reduction in free radical scavenging capacity ranging from 36 to 83 percent during storage. Moreover, Yang *et al.* (2007) [14] reported a decline in the antioxidant activity of noni juice.

Table 1: Changes in Bioactive compounds and antioxidant activity of Karonda Squash during storage

Storage Period	Bioactive compounds and antioxidant activity of Karonda Squash				
	Total Carotenoids (mg/100 g)	Total Phenols (GAE mg/100 g)	Total Flavonoids (RE mg/100 g)	FRAP (TE mg/100 g)	DPPH (TE mg/100 g)
0 Months	0.312	34.96	128.68	13.56	67.85
1 Month	0.306	34.46	127.03	13.15	67.48
2 Months	0.297	33.92	125.62	12.34	66.87
3 Months	0.287	33.36	123.77	11.41	63.84
4 Months	0.274	32.83	121.39	10.31	61.65
5 Months	0.261	32.22	116.83	8.63	57.43
6 Months	0.254	31.54	113.96	6.64	54.20
SEm±	0.001	0.36	1.04	0.15	0.39
C.D. at (5%)	0.004	1.11	3.17	0.46	1.20

Conclusion

The squash kept a lot of its biochemical composition and antioxidant capacity as shown by DPPH radical scavenging activity and FRAP though there is decline with increase in storage duration. The results revealed that it is possible to process Karonda fruit into squash.

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