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Short communication

Phytochemical studies on antioxidant activities of two types of *Karonda* (*Carissa carandas*) during storage

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ABSTRACT

***Carissa carandas* Linn. is a large dichotomously branched evergreen shrub. It has several medicinal and nutritional properties. An experiment was conducted considering two types, *viz.*, pinkish-green and pinkish-white to compare the phytochemical changes in perspective of antioxidant activity during storage. In fresh fruits, the maximum ascorbic acid content (50.98 mg/100 g) was recorded in pinkish green fruits as compared to pinkish-white (36.86 mg/100 g). Similarly, the scavenging activity of methanol extract of pinkish-green fruits was found significantly higher (89.57%) as compared to pinkish-white fruits (86.27%) in fresh samples. However, the minimum loss in phenol content (11.36%) was recorded in pinkish-white type during storage upto 8 days. Therefore, *karonda* fruits may be considered as an important phyto-chemical source for making different traditional medicines.**

Key words: Ascorbic acid, antioxidant activity, *Carissa carandas,* flavonoids.

Carissa carandas Linn. is commonly known as *karonda* (2n = 22) belongs to Apocynaceae family and found throughout India mainly in the semi-arid regions and have a long history of use in Indian traditional system of medicine. It is a very hard, drought tolerant plant that thrives well on a wide range of soils. The species is native to India and distributed in Sri Lanka, Indonesia, Malaysia, Myanmar and Pakistan. Fruits are generally harvested at immature stage for vegetable purpose, fully ripen fruits are consumed fresh or processed. Traditionally, this fruit is used for making pickles and sometimes candy like products commonly used in ice cream and bakery products as a substitute of cherry. It is a rich source of iron, therefore, in Uttar Pradesh, Uttarakhand and Rajasthan tribal people use for anaemic and pregnant women. It is generally used as live fencing border crops around field boundaries of fruit orchards, protecting crops from wild animals and also provides good returns to the farmers. *Karonda* fruits are the potential source of natural antioxidants. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extracts. Though, this fruit is traditionally used as a rich source of minerals, vitamins and phytochemicals but till date no systematic research has been carried out. Based on the above facts, the present study was undertaken on two types of *karonda* (green and white base) to compare the phytochemical changes with reference to antioxidant activity during storage.

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The mature fruits of *karonda* were collected from the field of Indian Institute of Farming Systems Research, Modipuram in the month of November, 2013 and 2014. At the time of fruit harvest, physiological parameters such as spade index and leaf area index were also measured with the help of SPAD-502 and canopy analyzer (LAI 2000), respectively. The harvested fruits were washed thoroughly with tap water and dried at room temperature for 2-3 h. The storage studies were conducted with fruits (pinkish-green and pinkish-white, 100 g each) containing 20-25 numbers having average weight of 4-6 g per fruit kept separately in different petri dishes and replicated three times. These fruits were kept in ambient condition (18 ± 2°C & 40 ± 5% RH) and analyzed for phytochemical properties, *viz.*, ascorbic acid content, total phenols, flavonoids and antioxidants at two days interval up to eight days in both the years.

Ascorbic acid content was determined by using the 2,6-dichlorophenol indophenol dye method suggested by Freed (4). The amount of ascorbic acid was calculated, and expressed as mg/ 100 g on a fresh weight basis. One gram of both type *karanda* were macerate in a mortar and pastel by using 10 ml methanol three times to extract the phytochemicals. The mixture was centrifuged at 9,000 rpm for 15 min. and the supernatant was decanted into a 50 ml volumetric flask for further chemical analysis. The amount of total phenolic contents in the extracts was determined calorimetrically with the Folin-Ciocalteu reagent, using a slightly modified method of Mansouri *et al.* (9). The results were expressed

as gallic acid equivalents (GAE)/100 g fresh weight. Total flavonoids content of the methanolic extract of *karonda* fruits was determined according to a modified colorimetric method (Zou *et al.,* 13). Briefly, 1.5 ml of fruit extract was taken and 75 µl of 5% $NaNO_2$ solution was added. After 6 min., 150 µl of 10% $AlCl_3.6H_2O$ was added to the mixture, which was kept at room temperature for 6 min. followed by the addition of 0.5 ml of 1M NaOH and the total volume was made up to 2.5 ml with the addition of deionised water. The resulting solution was mixed well and immediately, the absorbance was measured at 510 nm on a UV-VIS spectrophotometer. For the blank, the extracts were replaced with an equal volume of deionised water. A standard calibration curve was prepared with 0.01, 0.05, 0.1, 0.2, 0.4 and 0.6 mg/ml of quercetin (in deionised water). The total flavonoids content was expressed as the mg equivalents of quercetin (QE) per 100 g fresh weight. The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl- hydrazyl (DPPH)-free radical activity by modified method (Braca *et al*., 3). The optical density was recorded and per cent inhibition was calculated (Bors *et al*., 2). The total antioxidant activity of plant extract was also carried out using FRAP method (Benzie and Strain, 1). Data of 2013 and 2014 for quality parameters were subjected to analysis of variance (ANOVA), mean and standard deviation. Sources of variation were storage time and cultivars. All analysis was performed with a statistical software package SPSS v.11.0 for windows. Values of standard deviation are showed in figures.

At the time of harvest, the physiological parameters such as spade index and leaf area index were recorded. The spade index of *karonda* plants were recorded as 41.57 and 40.12 respectively for pinkish green and pinkish-white, whereas, leaf area index were recorded as 1072.11 and 831.45, respectively for pinkish-green and pinkish-white types.

Ascorbic acid content decreased during the course of storage in both the types of *karonda* (Fig.1). The ascorbic acid content was significantly higher in pinkish-green fruit in comparison of pinkish-white fruits. The ascorbic acid varied from 50.98 to 28.22 mg and 36.86 to 22.15 mg per 100 g for pinkish-green and pinkish-white base *karonda*, respectively during the storage upto 8 days. The ascorbic acid content decreased in pinkish-green and pinkish-white fruits follows the trend of linear equation y = -2.6384x + 48.045 and y = -1.88x + 37.864 with R^2 value 0.92 and 0.96, respectively. A rapid fall in ascorbic acid content (23.32%) was recorded in green fruits as compared to white one upto 2 days during storage (Fig. 1). However, the minimum ascorbic acid (39.90%) degradation was recorded in white fruits as compared to green fruits upto 8 days during storage. The ascorbic acid content decreased during storage may be due to the enzyme induced biochemical reactions. During storage, oxidizing enzymes like ascorbic acid oxidase, peroxidase, catalase and polyphenol oxidase might help in reducing the ascorbic acid of the fruits (Mapson,10). Whereas, vitamin C or ascorbic acid content in green *karonda* was reported 1.32 mg/g on dry weight basis (Jittawan *et al.,* 8). However, values of ascorbic acid content in *Karanda* were found very close to those values as reported by others (Haque *et al*., 5).

Further study shows that the total phenols also decreased from 19.73 to 14.48 mg and 15.84 to14.04 mg GAE per 100 g of green and white *karonda* fruits, respectively during storage. The decease trend of phenol follow the equation of y = -0.7659x +19.952 with R^2 value 0.81 and y = -0.2405x +15.858 with R^2 value 0.97 for green and white fruits, respectively. There was very minimum losses of total phenol content in case of white fruits, whereas 22.76% loss of phenol content was recorded upto 4 days of storage in green fruits. Though, the phenol content of green fruits was higher after harvest as compare to white types but the phenol content in white *karonda* more or less remain constant during 8 days of storage. After losing of 26.61% phenol content in green *karonda* upto 8 days of storage, the phenol content of both the fruits become at par (Fig. 2). It was reported that the levels of total phenolic compounds in the wild fruits varied significantly, from 1.3 to 214 mg gallic acid equivalent (GAE)/g dry weight, where the green based *karonda* contains 1.80 GAE/g dw (Jittawan *et al*., 8).

It was also found that flavonoid content was not significantly higher in green base than the white base types, but the flavonoids content decreased significantly during the storage of both type of *karonda*

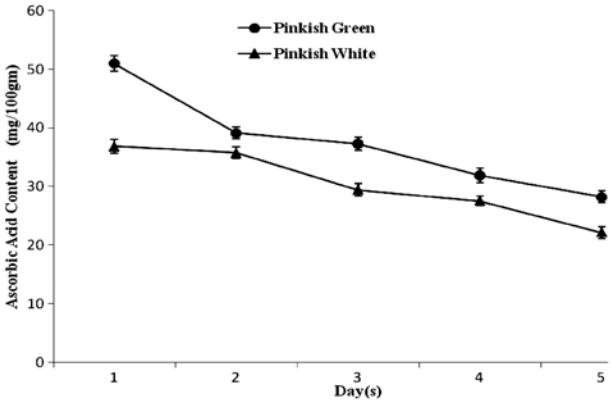

Fig. 1. Changes of Ascorbic acid content in *Carissa carandas* during storage.

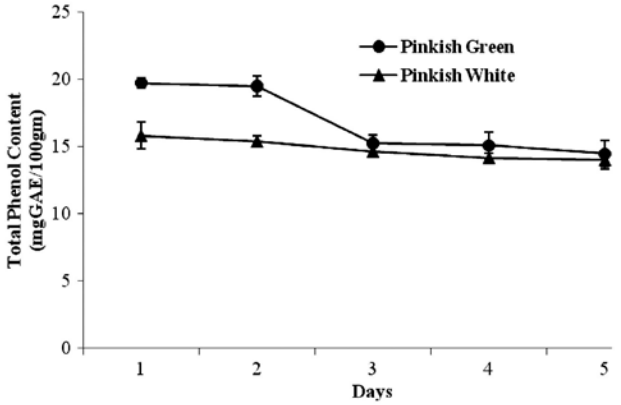

Fig. 2. Changes of total phenol content in *Carissa carandas* during storage.

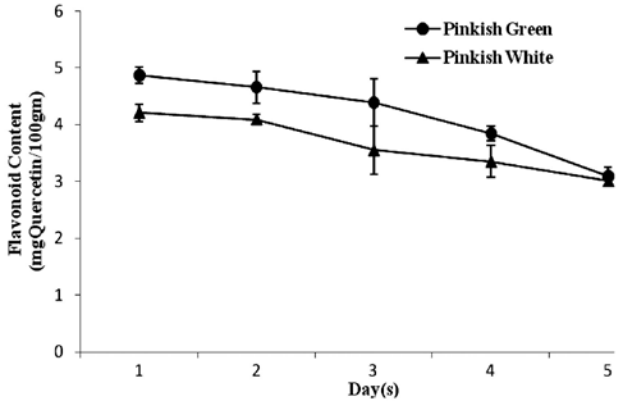

Fig. 3. Changes of total flavonoids content in *Carissa carandas* during storage.

as per the trend of y = -0.219x+5.046 and y = - 0.157x + 4.27, respectively. The flavonoids content vary from 4.87 to 3.09 mg and 4.21 to 3.01 mg quercetin equivalent mg/g of white and green fruits, respectively. Though, there was a decreasing trend of flavonoids during storage at ambient condition but the magnitude was different. It was found that the flavonoids content decreased by 9% in case of green fruits, whereas the flavonoid content decreased by 15% in case of white *karonda* upto 4 days of storage. After 8 days of storage, the flavonoids content decrease by 36% in green *karonda* as compared to the initial flavonoids content after harvest, whereas it decreased by 28% in white fruits and ultimately the flavonoids content became at par (Fig. 3) at 8 day of storage. Flavonoid content in the methanolic extract of *Carissa carandas* was found to be 2.92 rutin equivalents mg/g of extracts, which is nearly similar to the flavonoid content estimated in both types of *karonda* (Itankar *et al.*, 7). Besides their anti-oxidant activity, flavonoids have demonstrated a wide range of biochemical and pharmacological effects, including anti-inflammatory, anti-viral, anti-allergenic, anti-carcinogenic, anti-aging activity (Hulya, 6), anti-oxidant and anti-allergic effects (Miean and Mohamed, 11).

Different antioxidant compounds may act through different mechanisms and one method alone cannot be utilized to fully evaluate the antioxidant capacity. The antioxidant properties of green and white fruits of *Carissa* was estimated by DPPH and FRAP assays. The scavenging activity of methanol extract of *C. carandas* was significantly higher (89.57%) in case of green fruits compared to white fruits (86.27%). The per cent decrease of scavenging activity follow the trend of y = -0.789x + 89.862 with R^2 value 0.96 and y = -0.7605x + 86.6 with R^2 = 0.97 for green and white fruits, respectively during storage. The value of percent scavenging activity decease in similar pattern (Fig. 4) in both types during storage. The ferric reducing ability of plasma (FRAP) assay for antioxidant power was higher (13.93 mg GAE/g) in fresh green fruit extract than white fruits extract (12.91 mg GAE/g). In all the cases the antioxidant properties of green was higher than the white fruited *karonda* but the antioxidant properties were found gradually decreasing during the course of storage as per trend of linear equation y = -0.4215x + 13.874 with R^2 value 0.86 and y = -0.5655x + 13.068 with R^2 value 0.90, respectively. The value of FRAP assay decease in similar pattern (Fig. 5) in both type of *karonda* fruits during storage. Similar decrease in antioxidant content during storage was reported by (Nath *et al.*, 12) in case of broccoli florets. The deceasing trend of antioxidant properties may be due to the low value of polyphenol content in the sample during storage. There was positive correlation between the polyphenol content and

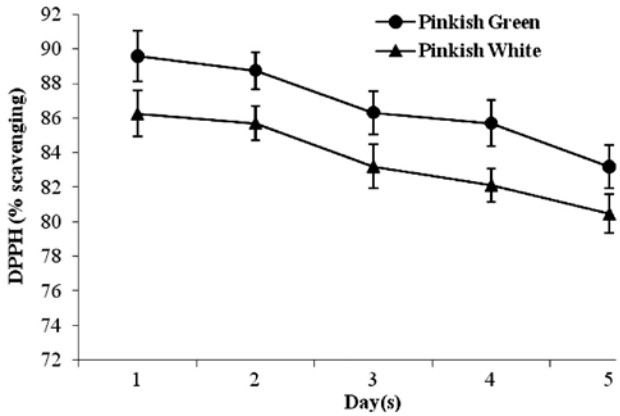

Fig. 4. Changes of scavenging activity of *Carissa carandas* during storage.

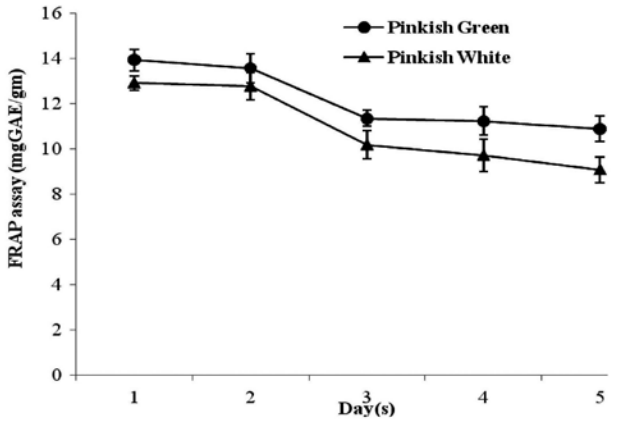

Fig. 5. Changes of ferric reducing ability of plasma (FRAP) in *Carissa carandas* during storage.

antioxidant activity of the fruit extract. Jittawan *et al*. (8) reported the variations in anti-oxidant activity values may be expected if multiple samples of each species had been harvested from different conditions of cultivation, location, ripening stage and season.

Green fruits of *Carissa* are enriched with antioxidant phytochemicals as compared to the white fruits. Though the market value of the white fruits of *Carissa carandas* is higher as compared to the green one, but from the nutritive value the green fruits of *Carissa carandas* is better. Therefore, green fruited *Carissa* may be explored as nutritive fruit for health conscious people since they have high ascorbic acid, phenol and flavonoids in addition to higher antioxidant properties.

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