

Antioxidant Activity and Nutraceutical Property of the Fruits of an Ethno-Medicinal Plant: *Carissa carandas* L. found in Brahmaputra Valley Agro-Climatic Condition.

Aniruddha Sarma*, Pranjal Sarmah, Debaleena Kashyap, Snehashish Dutta and Mantu Mahanta

*Institutional Biotech Hub,
Department of Biotechnology, Pandu College, Guwahati-12*

Abstract

Ethno-medicinally important plants or their parts have been used as a supplementary dietary source of nutrition and play a vital role in improving our health. Antioxidant compounds in diet play an important role as a health protecting factor. *Carissa carandas* L., an ethno-medicinally important plant, belongs to the family Apocynaceae and is an evergreen shrub or small crooked tree. It has been used in many ethno medicines as stomachic, anti-diarrheal and anti-helminthic and used in skin infections, fevers, rheumatism and biliary dysfunction. The present study was designed to evaluate the nutraceutical properties and antioxidant activity of the fruits of *C. carandas*. The antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and the IC₅₀ value of methanolic extract of *C. carandas* was found to be 27.45±0.43µg/ml. Among the nutraceutical properties carbohydrate (19.32 ± 0.69 mg/100g), protein (0.185 ± 0.011µg/g), crude fibre (15.64 % ±0.64), total phenolic content (188.75 ± 1.42 µgGAE/g) and ascorbic acid (62.93 ± 0.35 mg/100g) were recorded. The result shows that *C. carandas* have a high nutraceutical value. The study has revealed that the fruits can be used as dietary supplement as well as therapeutic agent as they would exert several beneficial effects by virtue of their antioxidant activity.

Keywords: *Carissa carandas*, Antioxidant activity, Total phenolic acid, Ascorbic acid, DPPH, Gallic acid.

1. INTRODUCTION:

Dietary habits are the habitual decisions of an individual or culture makes when choosing what food to eat and determined mainly by the availability of foods locally and local practices. A healthy diet may improve or maintain optimal health by giving an individual proper nutrition and protecting them from various diseases. Generally, nutrients are those substances which have health promoting as well as health protective activity. Broadly nutrients can be divided into two types- macro nutrients and micro nutrients. Macro nutrient includes protein, carbohydrate, lipid or fat and crude fibre. On the other hand, micro nutrient includes different minerals, vitamins, antioxidants etc. Reactive oxygen species (ROS) are generally produced during the various metabolic processes in our body. These ROS are involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with ageing¹. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, phenolic compounds, tocopherols and glutathione². When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage. It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds^{3, 4}. Phenolic compounds are potent antioxidants as they have beneficial effect on human health in fighting diseases by scavenging the reactive oxygen species (ROS)⁵. In India, many vegetables, fruits and plant based foods are known to have such health promoting and

protective properties. The plant *Carissa carandas* L. belongs to the family Apocynaceae and is an evergreen shrub or small crooked tree up to 3 m in tall with dichotomous branches armed with simple or forked, 2-4 cm long, paired axillary thorns. It is found naturally in the Himalayan region and also in the other parts of India. It flourishes well in the regions *C. carandas* has been used in many ethno medicines and has been traditionally used as stomachic, antidiarrheal and anthelmintic, stem used to strengthen tendons; fruits used in skin infections and leaves are remedy for fevers, earache, rheumatism, biliary dysfunction and syphilitic pain⁶. It is also consumed by the people as the raw or mature fruits are the most suitable for making pickle, jelly and candy. So it is of utmost importance to scientifically validate the medicinal and nutraceutical value of *C. carandas*. The present study was designed to evaluate the nutraceutical properties and antioxidant activity of the fruits of *C. carandas*.

2. MATERIALS AND METHOD:

The fruits of *Carissa carandas* L. were collected from the Barpeta district of Assam, India during the month of May to June. The location of Barpeta is 26.32°N 91.0°E with annual average rainfall 1,717mm, average temperature ranging from 18° to 38°C, humidity 76.6%. The collected samples were washed thoroughly, sliced and oven dried at 60 °C until they are completely dried and get constant weight. The dried slices were then powdered and kept at 4°C for further analysis. Chemical analysis was done on moisture free basis to estimate the carbohydrate content, protein content and crude fibre content, total phenolic content, ascorbic acid and antioxidant activity of the samples.

2.1 Total Carbohydrate Estimation:

The total carbohydrate content of the samples was estimated by Anthrone method⁷. 100 mg dried samples were hydrolyzed with 2.5 N HCl for about 3 hours in a boiling water bath. Sodium carbonate was added to neutralize the extracts. Subsequently the extracts were centrifuged and supernatant were collected. The residue was washed thrice with distilled water and all the supernatants were pooled and final volume was adjusted to 100ml. From this, 0.5 ml of the extracts was taken and volume made up to 1ml distilled water. 4ml of Anthrone reagent was added to the above solution. Absorbance was taken in a UV-Vis spectrophotometer at 630 nm and the amount of carbohydrate present was calculated by plotting the value in a standard curve of Glucose solution.

2.2 Total Protein Estimation:

The total protein content of the samples was estimated by following the method developed by Lowry *et. al.*⁸. Extraction is carried out with Tris-EDTA buffer (pH 7.5) used for the enzyme assay. 500 mg of the sample is grinded well with a mortar and pestle in 5ml of the buffer and after centrifuging, the supernatant is used for protein estimation. The reading is taken in a UV-Vis Spectrophotometer at 660nm and the amount of protein present is calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA).

2.3 Crude Fibre Content:

Crude fibre in the samples was determined by the method described by Maynard⁹. Defatted sample (2g) was placed in a glass crucible and attached to the extraction unit. 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in desiccator and weighed (W1). The sample was then ashed at 600°C in a muffle furnace (for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

$$\% \text{ crude fiber in ground sample} = \frac{\text{Loss in weight on ignition (W2 - W1)}}{\text{Weight of the sample}} \times 100$$

2.4 Total Phenol Content Estimation:

The total phenol content was determined by the Folin-Ciocalteu's method¹⁰. 200 µl of the fruit extracts (1 mg/ml) was taken and volume made up to 2 ml. 0.3 ml of Folin- Ciocalteu reagent was added. After 5 minutes, 0.8 ml of 20% Na₂CO₃ was added and the final volume was made 5 ml. Absorbance was taken by UV-Vis Spectrophotometer at 765 nm after 30 minutes incubation. The amount of phenol content was determined using Gallic

acid as standard. Results were expressed as µg/mg (Gallic acid equivalent/dry weight) and the calculations were done by using the following formula:

$$\text{TPC} = C \times V/m$$

Where, TPC= total phenol content, C= concentration of Gallic acid (mg/ml), V= volume of plant extract (ml) and m= weight of pure plant extract (g)

2.5 Ascorbic Acid Content Estimation:

The amount of ascorbic acid present in the samples was calculated by extracting the sample in 4% oxalic acid and titrating the extract against the 2, 6-dichloro phenol indophenol dye until the end point where pink colour appears that persist for a few minutes¹¹. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

$$\text{Amount of ascorbic acid} \left(\frac{\text{mg}}{100\text{g}} \right)_{\text{sample}} = \frac{0.5 \times V_1 \text{ ml} \times 100 \text{ ml}}{V_2 \text{ ml} \times 5 \text{ ml} \times \text{weight of the sample}} \times 100$$

Where, V₁= volume of oxalic acid, V₂= volume of the sample.

2.6 Antioxidant activity estimation:

The antioxidant activities of the fruit extracts along with standard were assessed on the basis of the radical scavenging effect of stable DPPH¹². A solution of DPPH of concentration 0.2 mM was prepared in 70% methanol and kept overnight. Stock solution (1 mg/ml) of the extract was prepared in 70% methanol. Various concentration of the extracts *viz.* 10, 20, 50, 100, 150, 200, 300, 400 and 500 µl were taken in different test tubes and the volume was made up to 1000 µl. 1 ml DPPH was added to each solution and kept at dark for 30 minutes. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1 ml methanol with 1 ml DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC₅₀) and calculated by the following formulae:

$$\text{Percent (\%) Inhibition of DPPH activity} = \frac{A - B}{A} \times 100$$

Where, A = Optical density of the blank
B = Optical density of the sample

2.7 Statistical Analysis:

The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as mean ± S.D. IC₅₀ value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

3. RESULT AND DISCUSSION:

The phytochemical analysis was done for total carbohydrate content, total protein content, crude fibre, total ascorbic acid content and total phenol content of *Carissa carandas* fruits (table 1). The antioxidant activity was measured using DPPH assay (fig. 1). In *Carissa carandas* fruits, the total phenol content was found to be 188.75 ± 1.42 µg GAE/mg whereas the ascorbic acid

content was 62.93 ± 0.35 mg/100 gm. The carbohydrate content was found to be 19.32 ± 0.69 mg/100gm and the protein content was 0.185 ± 0.011 µg/mg. The crude fibre content of the fruit was found to be $15.64 \pm 0.64\%$. The inhibition concentration (IC₅₀) of *Carissa carandas* was found to be 27.45 ± 0.43 µg/ml.

Medicinal plants are a major source of bioactive compounds of therapeutic values and are basis of many traditional medicines throughout the world. Herbal medicines are inexpensive, better compatibility with the human body and minimal side effects. The therapeutic value of a plant mainly depends upon its antioxidative property. Certain compounds like ascorbic acid, phenolic content, carotenoids, tocopherol etc. can enhance antioxidant activity. Findings of various researchers suggest that the phenolic compounds and ascorbic acid contributes greatly to the antioxidant activity of a fruit^{13,14,15,16}. Phenolic compound plays an important role for enhancement of antioxidant activity of certain medicinal plants like *Stellaria media*, *Persicaria chinensis* and *Olenlandia corymbosa*^{17,18}. These foods may provide an optimal mix of phyto-chemicals such as natural antioxidants and other bioactive compounds and thus protecting our body from various diseases. The present investigation suggests that the major source of antioxidant capacity of *Carissa carandas* is both ascorbic acid and phenolic compounds. Also the fruit is rich in nutritive constituents such as protein, carbohydrate, crude fibre, etc. Therefore, the fruits of *Carissa carandas* can be used as a source of natural antioxidant and can be supplemented through a balanced diet which could be much safer and cheaper than commercially available antioxidants.

ACKNOWLEDGEMENT:

The authors are very much thankful to DBT, Govt. of India for providing Instrumentation facilities under Institutional Biotech hub and Principal, Pandu College for permission and necessary laboratory facilities.

REFERENCE:

- Halliwell B and Gutteridge J M C. Free radicals in biology and medicine (3rd ed.)1999. Oxford: Oxford University Press.
- Mau J L, Chang C N, Huang S J and Chen C C. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculanta* and *Termitomyces albuminosus* mycelia. Food Chem 2004;87: 111-118.
- Cakir A, Ahmet Yildirim M A, Duru M E, Harmandar M and Kazaz C. Isolation and Characterization of antioxidant Phenolic Compounds from the Aerial Parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. J Ethnophar 2003; 87: pp. 73-83.
- Velioglu Y S, Mazza G, Gao L and Oomah B D: J. Agric. Food Chem 1998; 46: pp. 4113.
- Kalita Pallab, Barman Tapan K, Pal Tapas K, Kalita Ramen. Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug delivery & therapeutics 2013; 3(4): 33-37.
- N Balakrishnan and V H Bhaskar. Karaunda (*Carissa carandas* Linn.)- As a phytomedicine: A review. Kongposh publications 2009; pp.-95.
- Hedge J E and Hofreiter B T: In: *Carbohydrate Chemistry* 17 (Eds Whistler R L and Be Miller, J N) Academic Press New York, 1962.
- Lowry O H, Rosebrough N J, Farr A L and Randall R J: J Biol Chem 1951; 193: pp. 265.
- Maynard A J (Ed): Methods in Food Analysis. Academic Press New York; 1970; pp. 176.
- Singleton V L and Rossi JA. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16: pp. 144-158.
- Sadasivam S and Theymoli Balasubraminan: In: *Practical Manual in Biochemistr*. Tamil Nadu Agricultural University, Coimbatore 1987; pp. 14.
- Nooman A Khalaf, Khalaf N A, Shakya A K, Al-Othman A, El-Agbar Z and Farah H. Antioxidant activity of some common plants. Turk J. Biol 2008; 32: pp. 51-55.
- Prior R L, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G and Mainland C M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. J. Agric. Food Chem 1998; 46: pp. 2686-2693.
- Wang H, Cao G and Prior R L. Total antioxidant capacity of fruits. J. Agric. Food Chem. 1996; 44: pp. 701-705.
- Luximon-Ramma A, Bahoron T and Crozier A. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. J. Sci. Food Agri. 2003; 83: pp. 496-502.
- Sarma A, Sarmah P, Kashyap D and Kalita A. Evaluation of Nutraceutical properties and antioxidant activity of *Garcinia cowa* Roxb. ex Choisy fruits found in Assam (India). WJPPS 2014; 3(12): in press.
- Sarmah P, Sarma A, Kashyap D, Mahanta M and Medhi P. Nutraceutical properties of *Stellaria media* (L.) Vill. and *Persicaria chinensis* (L.) H. Grows under Brahmaputra valley agro-climatic condition, Annals of Plant Sciences 2014; 3(8): 779-782
- Sarmah P, Sarma A, Kashyap D, Kalita A and Choudhury S S. Nutraceutical properties and antioxidant activity of *Olenlandia corymbosa* L Grows under Brahmaputra valley agro-climatic condition. WJPPS 2014; 3(9): 586-592.