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# Estimation of total phenolic and flavonoid contents and evaluation of antioxidant activity of different parts of *Carrisa carandas* L

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**Abstract**
Plants are known as good sources of natural therapeutic agents. Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids and alkaloids *etc*. which reveal their specific characteristic properties and attribute to their pharmacological properties. Karonda are popularly consumed fruits in world because of its simple taste, high nutritional value and potential health benefits. In the present investigation, different parts of *Carrisa carandas* L. (fruits, seeds, stem, and leaves) were subjected to estimate total phenolic and flavonoid contents as well as extracts of these plant parts were also evaluated for their antioxidant activity by DPPH assay. Results of the present study indicated the presence of good quantity of total phenols and flavonoid contents in all the selected plant parts. The maximum phenolic content in while leaves of the plant were found to possess the highest phenolic content (28.96±4.26 mg QE/gdw) than other parts. Flowers were found to have the maximum flavonoid content (78.93±10.35mg GAE/gdw). The maximum free radical scavenging activity in was shown by leaves with the lowest $IC_{50}$ value (10.675 mg/L). All experiments were done in triplicates. One way ANOVA analysis showed lack of significant differences in antioxidant activity and total phenolic and flavonoid contents among all the selected plant parts.

**Keywords:** *Carrisa carandas, Total phenols,* total flavonoids, antioxidant activity etc

## Introduction

Various plants have been used for therapeutic purpose in different countries since ancient and are a good source of potent and powerful drugs. Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses (Petrovska, B., 2019) [13]. Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids and alkaloids *etc*. which reveal their specific characteristic properties and attribute to their pharmacological properties (Sudha, P. *et al*., 2011) [20]. The phytoconstituents have a major potential for developing phytomedicines which are considered to be generally safe.

As shown in recent years, natural antioxidants discovered in plants have attracted some interest due to their widely acclaimed nutritional and therapeutic values. Antioxidant properties stand to be an essential mechanism of beneficial activity of plant-derived compounds and extracts. Ethnopharmacological surveys have shed light on the fact that the therapeutic use of even 80% of 122 plant derived drugs may have a link with their recommendations in traditional medicine (Fabricant, D.S. *et al*., 2001) [5]. Natural antioxidants have a diversity of biochemical activities, some of which include the inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (Finkel, T. *et al*., 2000) [7]. Antioxidants have functioned to inhibit apoptosis because apoptosis was at first thought to be mediated by oxidative stress (Hockenbery, D.M. *et al*., 1993) [10]. It is known that many antioxidant substances have anticancer or anticarcinogenic properties (Johnson, I.T. *et al*., 1994; Dragsted, L.O. *et al*., 1993) [11, 3]. Epigallocatechin-3- gallate (EGCG) in green tea, for instance, has been reported to scavenge free radicals (Hanasaki, Y. *et al*., 1994) [9] and to hinder carcinogen-induced tumours in the skin, lung, forestomach, and colon of rodents (Stoner, G.D. *et al*., 1995) [17]. Therefore, there has been undeniable evidence of interest when it comes to finding natural antioxidants from plant materials.

Studies regarding the bioactivities of various plants have assumed an important position because of the variations in the effectiveness of the plant extract with the solvent for extraction used, plant part used, the plants' age, and geographic origin. The excessive use of medicinal plants for drug formulation also puts pressure on the need for more biomass of plants which can be met with biotechnological tools like micropropagation.

*Carissa carandas* Linn. is a fruit-bearing plant that grows as a tiny shrub in the Apocynaceae family that is widely distributed in subtropical and tropical regions and has been used for centuries as a medicinal herb in the Ayurvedic, Unani, and Homeopathic systems. In ethnomedicine, different parts of *C. carandas* have been used to treat anorexia, asthma, brain disease, constipation, cough, diarrhea, epilepsy, fever, leprosy, malaria, myopathic spams, pain, pharyngitis, scabies, and seizures. *C. carandas* has been observed to possess a wide spectrum of phytochemical constituents that varied in each part of the plant and result in various biological activities. Tannin, steroidal glycosides, phenolic compounds, and triterpenoidal constituents are abundant in the leaves. Several volatile compounds, as well as carissone and carindone, are present in the fruits while the seeds contain fatty acids such as linoleic acid, oleic acid, palmitic acid, and stearic acid. Therefore, all parts of *C. carandas* would be beneficial for human health and would have a potential to be used for anti-skin-aging.

In the present study, antioxidant activity along with total phenolic and flavonoid contents was evaluated in different parts of *Carrisa carandas* L. (fruits, seeds, leaves and stem) which belong to family Apocynaceae.

## Materials and methods

### Sample collection and processing

Plant samples, fruits, seeds, leaves and stem of *C carandas* L. were collected from University of Rajasthan, Jaipur, Rajasthan, India at different times. All samples were free from microbial and physical damage. The samples were washed, and the plant parts were separated. Those were shade dried at room temperature for 10 days. The dried parts were grounded in powdered form. The powered sample were stored in an airtight container separately and were kept being used for further phytochemical analysis.

### Sample extraction

The dried powered of plant parts were extracted by cold percolation method using methanol as a solvent. 10 g of the dried powder was taken in a conical flask having 100 ml methanol and kept in an orbital shaker at 120 rpm for 24 h. After 24 h, the extracts were filtered through Whatman filter paper no.1 for removal of peel particles and evaporated under vacuum.

### Determination of total phenolic contents in the plant extracts

TPC (The total phenolic content) was determined by the Folin-Ciocalteau method (Singleton, V.L. *et al*., 1965; McDonald, S. *et al*., 2001) [15, 12]. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml 7.5% $NaHCO_3$. The mixture was allowed to stand for 15 min at 45° C, and the phenols were determined by the spectrophotometric method. The absorbance was determined at λmax = 765 nm. The samples were prepared in triplicate, and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 100-1000 mg/L. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight), which is a common reference compound.

### Determination of total flavonoid concentrations in the plant extracts

The concentration of TFC (total flavonoid content) was determined using aluminium chloride spectrophotometric method (Chang, C. *et al*., 2002) [2] with slight modifications. Plant extracts (0.5 ml) were dissolved with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and incubated for half an hour at room temperature. The absorbance of the reaction mixture was measured at 415 nm. All experiments were prepared in triplicate, and the mean value of absorbance was obtained, and values were expressed in mean ± standard deviation. The standard curve was prepared using the standard solution of quercetin in methanol. Total flavonoid content of the extracts was expressed in milligrams of quercetin equivalents per gram dry weight.

### Extraction of flavonoids

Flavonoids were extracted from different parts of the selected plants following the well-established method of Subramanian and Nagarajan (1969). 100 grams of finely powdered plant parts were Soxhlet extracted with hot 80% methanol (500 ml) on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate. Each step was carried out three times to ensure complete extraction. Petroleum ether fraction was discarded due to being rich in fatty substances and ethyl ether fractions (free flavonoids) were collected. Ethyl acetate fractions were analysed for bound flavonoids. Each fraction was hydrolysed in 7% $H_2SO_4$ for 2 h. Resulting mixture was filtered and filtrate was again extracted with ethyl acetate. The ethyl acetate extract was washed with distilled water till neutrality and collected. The ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried i*n vaccuo*, weighed and stored in glass vials at 40° C till used.

### Determination of antioxidant activity

#### DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts were evaluated by 1, 1 - diphenyl 2 - picryl - hydrazil (DPPH) using the method given by Bhat and Karim (2009) [1]. An aliquot (100µl) of peel extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was vortexed thoroughly and kept in the dark for 30 min. The absorbance was measured at 515 nm, against a blank of methanol. The radical's scavenging activity was calculated using; $(Ab_{control} - Ab_{sample} / A_{control}) \times 100$ Where, $Ab_{control}$ is the absorption of the DPPH solution and Ab sample is the absorption of the DPPH solution after the addition of the sample. A linear graph of concentration vs percentage inhibition was prepared, and $IC_{50}$ values were calculated. The antioxidant activity of each sample was expressed in terms of $IC_{50}$ (defined as the amount of concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

### Statistical analysis

All experimental results were carried out in triplicate and were expressed as the average of three analyses with Standard Deviation. The $IC_{50}$ values were also calculated by linear regression analysis.

## Results and discussion

Plants contain many phytochemicals which are useful sources of natural antioxidants such as phenols, flavonoids, tannins, phenolic acids etc (Tungmunnithum, D. *et al*., 2018) [21]. Various studies have been reported that presence of good phenolic and flavonoid contents in plants are indicator of their antioxidant capacity. Flavonoids are a widely distributed group of phenols that act as effective antioxidants. Being plant secondary metabolites, the phenolics or polyphenols are very important judging from the virtue of their antioxidant activities by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydroperoxide conversions into reactive oxyradicals (Esmaeili, A.K. *et al*., 2015) [4]. Results of the present study revealed that both the selected plant parts are good sources of phenolic and flavonoid contents (Table 1). The maximum phenolic content in was found in leaves (28.96±4.26 mg GAE/gdw) compared to fruits, seeds, and stem while fruits of the plant were found to possess the highest flavonoid content (78.93±10.35 mg QE/gdw) than other parts. Free and bound flavonoids were also extracted from plants and from all plant parts, good amount of both types of flavonoids were isolated. Result of one-way

ANOVA showed lack of significant differences in phenolic and flavonoid contents in various plant parts and between different species. Presence of good amount of phenolic and flavonoid contents showed that all the species are good sources of natural antioxidants as the rich-flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement of the overall antioxidant capacity of an organism and protection against lipid peroxidation (Sharififar, F. *et al*., 2009) [14].

The polyphenol anti-oxidant capacity has been taken into account as one of the outstanding mechanisms of action in inhibiting mutagenesis and cancer initiation by means of their capacity to scavenge ROS, activate antioxidant enzymes, prevent carcinogen-induced DNA adduct formation, enhance DNA repair and reduce overall oxidative DNA injury (Stoner, G.D. *et al*., 2008) [18]. DPPH is commonly used assay to evaluate free radical scavenging activity of plant extract. In the present investigation, ethanolic extracts of the selected plant parts were subjected to DPPH assay at different concentrations (0-100 mg/L) and results showed that the selected parts of the selected plant have great potential to scavenge free radicals (Table 2, Figure 1). The maximum free radical scavenging activity in *Carrisa carandas* L. was shown by leaves with the lowest $IC_{50}$ value (10.675 mg/L). No significant difference was found in $IC_{50}$ values of different parts, and in different species by one way ANOVA analysis.

**Table 1:** Total phenolic, total flavonoids, and isolated flavonoid contents in different parts of *C. carandas* L

| Plant part | Total phenolic content (mg GAE/gdw) | Total flavonoid content (mg QE/gdw) | Isolated flavonoids (mg/g. dw) | | |
|---|---|---|---|---|---|
| | | | Free (F) | Bound (B) | Total (F+B) |
| Fruits | 21.58±3.72 | 78.93±10.35 | 1.858 | 0.994 | 2.852 |
| Seeds | 4.58±1.15 | 15.64±1.58 | 0.528 | 1.059 | 1.587 |
| Leaves | 28.96±4.26 | 64.52±12.87 | 2.842 | 0.616 | 3.458 |
| Stem | 11.52±1.63 | 22.85±5.97 | 2.784 | 0.341 | 3.125 |

**Table 2:** Evaluation of antioxidant activity of plant parts of *C. carandas* L. by DPPH assay

| Plant part | % Scavanging activity at different concentration | | | | | Regression equation | $IC_{50}$ value (µg/ml) |
|---|---|---|---|---|---|---|---|
| | 20 µg/ml | 40 µg/ml | 60 µg/ml | 80 µg/ml | 100 µg/ml | | |
| Leaves | 7.41 | 13.82 | 19.46 | 23.34 | 23.78 | Y=4.226x+4.884 | 10.675 |
| stem | 20.57 | 23.67 | 25.55 | 26.76 | 30.53 | Y=2.301x+18.513 | 13.684 |
| seed | 19.35 | 21.23 | 21.46 | 24.88 | 29.64 | Y=2.423x+16.043 | 14.014 |
| fruit | 16.26 | 19.13 | 21.46 | 24.88 | 29.64 | Y=3.251x+12.521 | 11.528 |

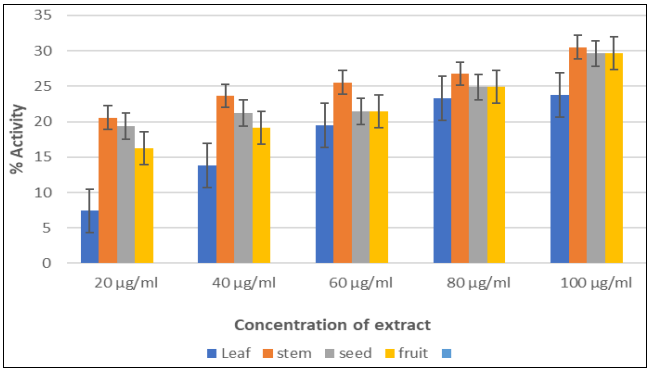


**Fig 1:** Free radical scavanging activity by different plant parts of *C. carandas* L.

## Conclusion

The present work shows the presence of high flavonoid and phenolic contents in different parts of different parts of *Carrisa carandas* L. (fruits, seeds, leaves and stem) which is common edible plant. Results of the study further suggested that these plant parts are rich source of natural antioxidants and eating karonda fruit provide good antioxidants and can protect us from various diseases. The present study may help researchers and scientists to make strategies of developing antioxidant rich varieties of food crops.

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