

IN VITRO ANTIOXIDANT ACTIVITY OF CHLOROFORM AND ETHANOLIC FRUIT AND ROOT EXTRACTS OF *CARISSA CARANDAS* LINN.

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ABSTRACT

Carissa carandas (L.) belonging to the Apocynaceae family and it is represented about 89 species in India. Many plants of this family are the sources of important constituents of therapeutic importance. It naturally grows in the Himalayas at a height of 300 to 1800 meters in the Siwalik Hills from sea level and require fully exposure to sun and unfavorable to humidity. Out of the 8 Indian species, 3 are of economic importance and good medicinal values. The high value of total phenolic and total flavonoid content in the CFCC, CRCC, EFCC and ERCC may be responsible for its free radical scavenging activity. DPPH is a stable free radical at room temperature and accepts one electron or hydrogen radical to become a stable diamagnetic molecule. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl (-OH) groups. The antioxidant activities of the chloroform, ethanolic fruit and root extracts of *C. carandas* may be probably due to the presence of compounds with hydroxyl groups.

Keywords: *Carisaa carandas*, flavonoid, antioxidant, hydroxyl groups

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INTRODUCTION

Carissa carandas (L.) belonging to the Apocynaceae family and it is represented about 89 species in India. Many plants of this family are the sources of important constituents of therapeutic importance [1]. It naturally grows in the Himalayas at a height of 300 to 1800 meters in the Siwalik Hills from sea level and require fully exposure to sun and unfavorable to humidity [2]. Out of the 8 Indian species, 3 are of economic importance and good medicinal values [3]. In folklore medicine, the fruit extracts of *C. carandas* Linn are traditionally used as anti-diarrheal and intermittent fever [4], Anthelmintic, astringent, appetizer, antipyretic, anti-diabetic, aphrodisiac, in biliary disorders, stomach disorders, antioxidant, skin diseases [5-6]. While, root extracts for Anthelmintic, scabies, wound healing, intestine worms, ulcer and purities and anti-diarrheal [7-8], this is because of availability of Phytoconstituents.

Antioxidants are vital substances which possess the ability to protect the human body from damages caused by free radical induced oxidative stress. Free radicals are fundamental to any biochemical methods and represent an essential part of aerobic life and metabolism [9]. Polyphenolic compounds constitute an important category of antioxidant metabolites. The Phenolic substances such as flavonols, cinnamic acids, coumarins and chlorogenic acids or caffeic acids believed to have antioxidant properties and which are suggested to play an important role in protecting

Vegetables, food, cells and any organ from oxidative degradation [10-12]. Therefore, the search for natural antioxidant has greatly increased year by year. In the present study, the ethanolic fruit and root extracts of *C. carandas* were screened for antioxidant properties using *in vitro* standard procedures so as to assess exact medicinal potential of the plants and justify their folklore uses.

Materials and Methods

Chemicals

Butylated hydroxytoluene (BHT), L- ascorbic acid, gallic acid, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitropruside, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), trichloroacetic acid (TCA) and ferric chloride were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant Materials

The dried powdered of fruit of plant material (500gm) and roots of *C. carandas* (500gm) were subjected into hot successive extraction in a Soxhlet apparatus with Chloroform and ethanol solvents. The solvents should be evaporated with the help of rotary vapor then extracts chloroform and ethanolic fruit extracts of *C. carandas* (CFCC and EFCC) and chloroform and ethanolic root extracts of *C. carandas* (CRCC and ERCC) were collected and calculate the yield values of each extracts [13].

Phytochemical Screening

The various test performed for identification of different Phytochemical constituent like; alkaloids, flavonoids, glycosides, carbohydrates, sterol, terpenoid, tannins and saponin etc which is depending upon the type or nature of the crude drugs [13-18].

Determination of total phenol

Total phenolic contents (TPC) in the ethanolic fruit and root extracts were determined by the using the Folin-Ciocalteu method. A diluted solution of each ethanolic extracts (0.5 ml of 1:10 g ml⁻¹) or gallic acid (Standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and sodium carbonate (4 ml, 1M). The mixtures were allowed to stand for 15 min and then determined the total phenols by using UV-VIS spectrometer at 765 nm and the content expressed in terms of gallic acid equivalent (mg/g) [19].

Determination of total flavonoid

Aluminum chloride colorimetric method was used for determination of flavonoids. Fruit and root extracts of plant (0.5 ml of 1:10 g ml⁻¹) in methanol was separately mixed with 1.5 ml of 1M potassium acetate and 2.8 ml of distilled water. It was maintained at room temperature for 30 min and then the absorbance of the mixture at 415 nm was measured with UV-VIS spectrometry and the total flavonoids content (TFC) was expressed in terms of catechin equivalent (mg/g) [20].

Determination of DPPH radical Scavenging activity

The free radical scavenging activity of CFCC, CRCC, EFCC, ERCC and butylated hydroxyl toluene (BHT) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. DPPH solution (0.1 mM) in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract solution in water at different concentrations (10-300 µg/ml). After 35 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [21].

Determination of NO radical scavenging activity

Nitric oxide (NO) was generated from Sodium Nitroprusside and measured by the Greiss reaction. Aqueous solution of Sodium nitroprusside at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions and that can be estimated by use of Greiss reagent [22]. Scavengers of NO compete with oxygen leading to decrease the production of NO. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3 ml of different concentrations (10 –300 µg/ml) of the CFCC, CRCC, EFCC, ERCC and BHT dissolved in the suitable solvent systems and incubated at 25 °C for 2 hr and 30 min. The extract samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% Naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite

with sulphanilamide and subsequent coupling with Naphthylethylenediamine was observed at 546 nm. The NO radicals

scavenging activity was calculated as in the case of DPPH [23].

Table 5.13: Total phenolic and flavonoid content of fruit and root extracts of *C. carandas*

Group	Total phenolic content (TPC) ¹	Total flavonoid content (TFC) ²
CFCC	14.32 ± 0.2538	2.25 ± 0.1000
CRCC	11.21 ± 0.0200	1.12 ± 0.0862
EFCC	17.10 ± 0.2683	2.69 ± 0.0896
ERCC	14.15 ± 0.3000	1.42 ± 0.0873

Values represent mean ± SEM (n=3)

¹ Expressed as mg of gallic acid equivalents / g of dry plant part extract

² Expressed as mg of catechin equivalents / g of dry plant part extract

Table 5.14: IC₅₀ value of standard (BHT), fruit and root extracts of *C. carandas* in DPPH and Nitric oxide assay

<i>C. carandas</i> (fruit and root fractions)	IC ₅₀ (µg/ml) DPPH	IC ₅₀ (µg/ml) Nitric oxide
BHT	64.52	88.68
CFCC	237.40	279.92
CRCC	259.59	298.33
EFCC	195.81	219.92
ERCC	216.75	211.25

Data code indicated as; BHT: Butylated hydroxytoluene, CFCC, Chloroform fruit extract of *C. carandas*; CRCC, Chloroform root extract of *C. carandas*; EFCC, Ethanolic fruit extract of *C. carandas*; ERCC, Ethanolic root extract of *C. carandas*.

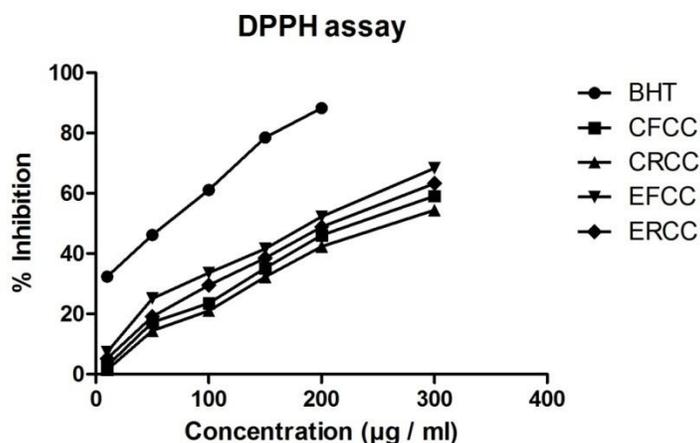


Figure 1. IC₅₀ value of standard (BHT), fruit and root extracts of *C. carandas* in DPPH Assay

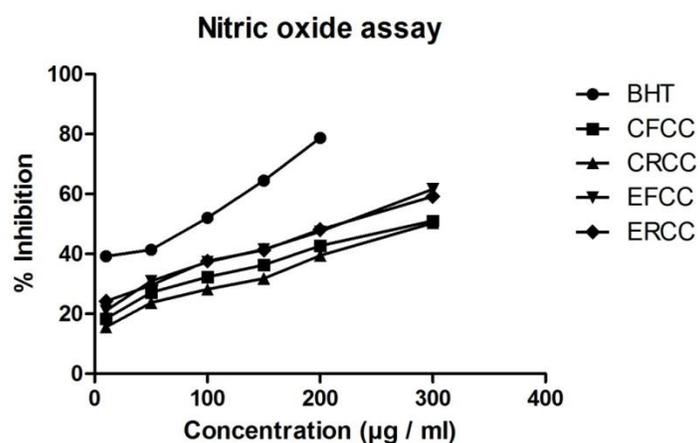


Figure 2. IC₅₀ value of standard (BHT), fruit and root extracts of *C. carandas* in Nitric oxide Assay

Results

The Phytochemical screening reveals the presence of Phytoconstituents like; alkaloids, Flavonoids, glycosides, reducing sugar, steroids, terpenoid and tannins are present in ethanolic fruits and root extracts of plant. While Flavonoids, sterols, terpenoid and tannins are present in chloroform fruit and root extracts of plant, which is best proved for the confirmation about the plant and expected different traditional use and the various pharmacological activities.

The Acute toxicity studies of selected fruit and root extracts showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. Hence there is no LD₅₀ and all the extracts tested are considered safe and nontoxic. During Antioxidant activity, the content of total phenolic (determined using Folin-Ciocalteu assay) and flavonoids in CFCC, CRCC, EFCC and ERCC were significant and observed in Table 1. EFCC and ERCC had higher amounts of total flavonoid and

phenol content than CFCC and CRCC. The antioxidant properties of CFCC, CRCC, EFCC, ERCC and BHT led to observed with the help of DPPH and Nitric oxide Assay of the fruit and root extracts of the plant. The IC₅₀ (µg/ml) DPPH radical values of BHT, CFCC, CRCC and EFCC, ERCC are 64.52, 237.40, 259.59, 195.81 and 216.75 respectively. The IC₅₀ (µg/ml) Nitric Oxide of BHT, CFCC, CRCC and EFCC, ERCC were 88.68, 279.92, 298.33, 219.92 and 211.25 respectively and shown in Table 2. The extracts and BHT compete with oxygen to react with NO and thus inhibit the peroxy nitrite formation.

Discussion

The high value of total phenolic and total flavonoid content in the CFCC, CRCC, EFCC and ERCC may be responsible for its free radical scavenging activity. DPPH is a stable free radical at room temperature and accepts one electron or hydrogen radical to become a stable diamagnetic molecule [24]. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl (-OH) groups. So, it is concluded that, the antioxidant activities of these chloroform, ethanolic fruit and root extracts of *C. carandas* may be probably due to the presence of compounds with hydroxyl groups.

NO is involved in the regulation of various physiological processes were excess concentration of NO is associated with various types of diseases and ailments. Oxygen reacts with the excess of NO to generate nitrite and peroxy nitrite anions, which act as free radicals. This result

suggested that the phenolic components are present in the chloroform, ethanolic fruit and root extract might be responsible for NO Scavenging effects.

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