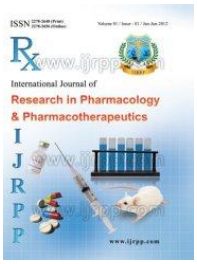
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.5 | Issue 1 | Jan- Mar - 2016***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

In vitro antioxidant and free radical scavenging studies of *Carissa carandas L.*

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ABSTRACT

Free radicals are implicated in many diseases, including cancer, diabetes mellitus, arthritis, ageing. etc., In the treatment of these diseases, antioxidant therapy has gained very importance. Various extracts of *Carissa carandas L* were studied for its *in vitro* antioxidant activity used different models viz. ABTS radical scavenging, DPPH radical scavenging, H_2O_2, Hydroxyl radical, lipid peroxidation and Super oxide. The results were analyzed statistically by the regression methods. The scavenging effect of plant extracts and standardized (L. ascorbic acid) on the various scavenging methods in the following order L-ascorbic acid >Ethanol>Ethyl acetate>Chloroform>Pet ether. Its antioxidant activity was estimated by IC_{50} values and the values are 124.62 TO276.42µg/ml (ABTS radical scavenging), 205.24TO 354.52 µg/ml (DPPH radical scavenging), 168.12 TO 262.48 µg/ml (Hydrogen peroxide), 204.06 to 292.44 µg/ml (Hydroxyl radical), 154.02 to 310.22 µg/ml (Lipid peroxidation), 161.84 to 360.44µg/ml (Super oxide). In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals, metal chelation or inhibition of the liquid peroxidation. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. The results clearly indicate that *Carissa carandas L* is effective against free radical mediated disease.

Keywords: ABTS, DPPH, H_2O_2, Hydroxyl radical, Lipid peroxidation, Super oxide, *Carissa carandas.L*

INTRODUCTION

Medicinal herbs constitute effective sources of antimicrobial, anticancer and antioxidant natural products (Calixto, B.J., 2000) Medicinal herbs are an important source for the therapeutic remedies for various ailments (Doss, A. and S.P. Anand, 2012.)Antioxidants are substances that prevent damage to cells caused by free radicals and search for free radicals, lend them electron which stabilizes the molecule, thus preventing damage to other cells.

Antioxidants also turn free radicals into waste byproducts, and they eventually get eliminated from the body. They also have the ability to repair previous damage to cells. These antioxidants are found naturally in fruits and vegetables.

Caranda is a shrub growing 2 to 3 meters high. Branches are numerous, rigid and spreading, with 2 straight, simple or forked thorns, up to 5 centimeters in length on the axils and nodes. Leaves are smooth, ovate, or oblong-ovate, 4 to 7.5 centimeters in length, 2.5 to 4 centimeters in width, rounded or notched at the base, and blunt tipped.

Flowers are fragrant, white or pale rose-colored, clustered in twos or threes. Calyx-segments are very slender, pointed and hairy. Corolla tube is about 2 centimeters long, smooth, with a swollen throat and hairy lobes, the lobes being lanceolate, pointed, spreading and about half as long as the tube. Fruit is a drupe, broadly ovoid, 1.5 to 2.5 centimeters long, bluntly pointed, and blackish or reddish-purple, containing 2 to 4 small, flat seeds. Pulp is reddish-purple and sour.

The purpose of this study was to evaluate *Carissa carandas L* as a potential source of natural antioxidants.

MATERIAL AND METHODS

All chemicals and solvents were of analytical grade and were of obtained from Ranbaxy Fine Chemicals, Mumbai, India.

Plant material

The fresh aerial parts of *Carissa carandasL (Apocynaceae)* were collected from the local region of Bhavani, Tamil Nadu, India in June 2012and were authenticated by and were authenticated by Prof. P. Jayaraman, Ph.D, Director, Plant Anatomy Research Center, Chennai-5.

Plant extract

About 500 g of the crude powder was taken and extracted in a soxhelet extractor with various solvents such as petroleum ether, chloroform, ethyl acetate and ethanol (2 Lit.). The crude extract was concentrated to dryness in rotary flash evaporator under reduced pressure and controlled temperature (40-50 ^{0}C). The extract was preserved in vacuum desiccators for subsequent use in the study.

ABTS radical scavenging assay

To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 2 to 500 µg/ml. Blank was carried out without drugs. Absorbance of recorded at 734 nm. An experiment was performed in triplicate (Sreejayan, 1996, John, 1984).

DPPH radical scavenging assay

To the methanolic solution of DPPH (1 mm) an equal volume of the extracted dissolved in alcohol was added at various concentrations from 2 to 1000 µg/ml in a final volume of 1.0 ml. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. An experiment was performed in triplicate (Sreejayan, 1996, John, 1984).

The scavenging activity of extract towards Hydrogen peroxide radicals was determined by this method (Houghton *et al*, 2005). 2ml of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4) and 1.0 ml of methanolic sample (5-1000µg/ml of extract of plant /standard ascorbic acid) was added to hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The experiment was repeated in triplicate.

Hydroxyl radical scavenging activity *p*-NDA method. Various concentrations of the extract / Standard of ascorbic acid (5-1000µg/ml) or standards in 0.5 ml of distilled DMSO were added to a solution mixture containing 0.5 ml of ferric chloride (0.1 mm), 0.5 ml of EDTA (0.1 mm), 0.5 ml of ascorbic acid (0.1 mm), 0.5 ml of hydrogen peroxide (2 mm) and 0.5 ml of *p*-NDA (0.01 mm) in phosphate buffer (pH 7.4, 20 mm) to produce a final volume of 3 ml. Absorbance was measured spectrophoto metrically at 440 nm. All the measurements were carried out triplicate.

ABTS radical scavenging assay

To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 2 to 500 µg/ml. Blank was carried out without drugs. Absorbance of recorded at 734 nm. An experiment was performed in triplicate (Sreejayan, 1996, John, 1984).

Lipid peroxidation assay

The mixture (Egg phosphatidylcholine in 5 ml saline) was sonicated to get a homogeneous suspension of liposome. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid to a mixture containing liposome (0.1 ml). The pink chromogen was extracted with a constant volume of n-butanol and absorbance of the upper organic layer were measured at 532 nm. The experiment was performed in triplicate (Sudheerkumar, 2003).

Nitric oxide radical scavenging

Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test compound, Sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ^{0}C for 5 hours, after which, 0.5 ml

of Griess reagent was added. The absorbance of chromophore was read at 546 nm. An experiment was performed in triplicate (Sreejayan, 1996).

Superoxide scavenging

Alkaline DMSO was used as a super oxide generating system. To 0.5 ml of different concentrations of the test compounds, 1 ml of alkaline DMSO and 0.2 ml of NBT 20 mm in phosphate buffer pH 7.4 was added. An experiment was performed in triplicate (Govindarajan, 2003).

Statistical analysis

All results are expressed as mean ± S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC_{50} values.

RESULTS AND DISCUSSION

Several concentrations ranging from 2 to 1000 µg/ml of the alcoholic extract of *Carissa carandas L* were tested for their antioxidant activity in different *in vitro* models. It was observed that free radical was scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values were calculated for all models given in table 1.

Table 1. *In vitro antioxidant* activity of *Carissa carandas L* extractsIC_{50} values ± SE (µg/ml)

Extract	ABTS	DPPH	H_2O_2	HYDROXYL RADICAL	LIPID PEROXIDATION
Pet ether	276.42 ±0.28	354.52±2.82	262.48±0.26	292.44±0.36	310.22±0.28
Chloroform	204.21±0.32	306.48±2.84	231.62±0.14	286.12±0.42	204.16±0.42
Ethyl acetate	165.32±0.44	218.32±3.06	170.72±0.42	210.12±0.32	182.08±0.06
Ethanol	124.62±0.54	205.24±3.12	168.12±0.198	204.06±1.42	154.02±1.02
Standards Ascorbic acid	102.4±0.15	202.64±3.02	149.92±0.13	181.08±1.35	136.08±0.08

Oxidative stress has been implicated in the pathology of many diseases and conditions includes in diabetes, cardiovascular diseases, inflammatory conditions, cancer, and ageing (Joyce, 1987, Velioglu, 1998). The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm (Ganapathy, 2007)

The antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies (Rice-Evans, 1997)

Initiation of the lipid peroxidation by ferrous sulphate takes place either through the ferryl-perferryl complex or through OH radical by Fenton's reaction. Thus the decrease in the MDA level in egg phosphatidylcholine with the increase in the concentration of the extract indicated the role of the extract as an antioxidant

Superoxide can cause oxidation or reduction of solutes depending on the reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyses the breakdown of superoxide radical (Shirwaiar, 2007). In our study, alkaline DMSO used for superoxide generation indicates that *C.carandas L* is a potent superoxide scavenger.

CONCLUSION

C. CarandasL an evergreen, deciduous shrub with immense medicinal value has been revived with the aim to provide a reference source for biology, photochemistry, ethnopharmacology, and conservation strategy for further research on the plant. The results of the present study show that the extract of *Carissa carandas L* contains the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing.

Acknowledgement

The authors sincerely thank J.K.K.M.M.R, F's Annai J.K.K. Sampooraniammal College of Pharmacy, Komarapalayam, Tamilnadu for providing the necessary facilities to carry out this research work.

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