



International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR | Volume 4 | Issue 1 | Jan – Mar - 2016
www.ijamscr.com

Research article

Pharmacological research

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

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ABSTRACT

Free radicals are implicated for many diseases including cancer, diabetes mellitus, arthritis, ageing etc. In the treatment of these diseases, antioxidant therapy has gained very importance. Various extract of *Carissa spinarum L* was studied for its in vitro antioxidant activity used different models viz. ABTS radical scavenging, DPPH radical scavenging, H₂O₂, Hydroxyl radical, lipid peroxidation and Super oxide. The results were analyzed statistically by the regression methods. The scavenging effect of plant extracts and standard (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 IC₅₀ value and the values are 102.6 to 244.2 µg/ml (ABTS radical scavenging), 200.41 to 354.42 µg/ml (DPPH radical scavenging), 152.31 to 236.12 µg/ml (H₂O₂), 188.74 to 296.41 µg/ml (Hydroxyl radical), 142.12 to 291.41 µg/ml (Lipid peroxidation), and 157.42 to 306.16 µg/ml (Super oxide). The results clearly indicate that *Carissa spinarum L* has a significant potential to use as a natural antioxidant agent.

Keywords: ABTS, DPPH, H₂O₂, Hydroxyl radical, Lipid peroxidation, Super oxide, *Carissa spinarum L*

INTRODUCTION

According to WHO (1993) 80% of the world's population is dependent on the traditional medicines and a major part of the traditional therapies involves the use of plant extract or their bioactive compounds (Mathur A, 2011). Plant – based natural constituents can be derived from any part of plant (Gordon M C and David 2001). 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 of 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 by products, 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.

C spinarum L (Apocynace) 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 (Malik SK, 2010). Calyx-segments are very slender, pointed and hairy.(Maheswari R, 2012) 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 spinarum L* as a potential source of natural antioxidants.

MATERIAL AND METHODS

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

Plant material

The fresh aerial parts of *Carissa spinarum L* (Apocynaceae) were collected from the local region of Bhavani, Tamil nadu India in November 2011 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 Soxhlet 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 °C). The extract was preserved in vacuum desiccators for subsequent use in 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 was recorded at 734 nm. 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 extract 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. Experiment was performed in triplicate (Sreejayan, 1996, John, 1984).

The scavenging activity of extract towards Hydrogen peroxide radicals was determined by this method (Subin Mary Zachariah et al, 2012). 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. Various concentration 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 spectrophotometrically at 440 nm. All the measurements were carried out in triplicate. (Babu et al., 2001)

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 was measured at 532 nm. The experiment was performed in triplicate (Sudheerkumar, 2003).

Superoxide scavenging

Alkaline DMSO was used as a super oxide generating system.(Shirwaiar,2007) 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. Experiment was performed in triplicate (Govindarajan, 2003).

Statistical analysis

All results are expressed as mean \pm S.E.M. Linear regression analysis (Origin 6.0 version) was used to calculate the IC₅₀ values.

RESULTS AND DISCUSSION

Several concentrations ranging from 2 to 1000 μ g/ml of the various extract of *Carissa spinarum 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 IC₅₀ values were calculated for all models given in table 1.

Table 1. *In vitro* antioxidant activity of *Carissa spinarum* extracts IC₅₀ values \pm SE (μ g/ml)

Extract	ABTS	DPPH	H ₂ O ₂	Hydroxyl radical	Lipid peroxidation	Super oxide
Pet ether	244.2 \pm 0.36	354.42 \pm 2.02	236.12 \pm 0.22	296.41 \pm 0.28	291.41 \pm 0.16	306.16 \pm 0.12
Chloroform	178.4 \pm 0.42	302.38 \pm 3.12	212.38 \pm 0.32	285.32 \pm 0.41	188.42 \pm 0.22	202.16 \pm 0.38
Ethyl acetate	136.21 \pm 0.16	212.36 \pm 2.02	168.12 \pm 0.24	216.42 \pm 0.26	164.18 \pm 0.42	169.38 \pm 0.42
Ethanol	102.6 \pm 0.22	200.41 \pm 2.68	152.31 \pm 0.46	188.74 \pm 1.62	142.12 \pm 0.12	157.42 \pm 0.68
Ascorbic Acid	99.8 \pm 0.16	198.32 \pm 2.82	136.29 \pm 0.52	168.92 \pm 1.87	128.28 \pm 0.06	128.92 \pm 0.82

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 (Sudheerkumar, 2003)

Superoxide can cause oxidation or reduction of solutes depending on the reductions potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalysis the

breakdown of superoxide radical (Shirwaiar, 2007). In our study, alkaline DMSO used for superoxide generation indicates that *C.spinarum L* a potent superoxide scavenger

CONCLUSION

C.spinarum Lan ever green, 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 spinarum 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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How to cite this article: K.Sumathi, N.Senthil Kumar, In vitro antioxidant and free radical scavenging studies of carissa Spinarium L. *Int J of Allied Med Sci and Clin Res* 2016; 4(1): 108-111.

Source of Support: Nil. **Conflict of Interest:** None declared.