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EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF STEM EXTRACT OF CARISSA CARANDAS

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ABSTRACT

Carissa carandas Linn., also referred to as the Karonda christ hawthorn since it grows naturally in bushes. In India's tropical and subtropical Adriatic region, it is only occasionally grown. It is a popular medicinal herb in numerous indigenous medical practises, notably Unani, Ayurveda, and homoeopathy, and is utilised frequently by Indian tribal people. It collects little berries that are widely used to season and preserve Indian pickles and spices. In addition to Karonda karonda (Devanagari: karamardaka (Sanskrit), Koromcha (Bengali), Christ's thorns (South India), vakkay (Telugu), kilaakkaai (Tamil), and Karja tenga (Assamese), the prickly plant is also known by other names.), among others. The study focused on investigating the antidiabetic and antioxidant activities of *Carrisa Carandas* Linn. through the use of ethanol stem extract. Preliminary studies and GC-MS study revealed the presence of various phytochemical group like carbohydrates, amino acids, alkaloids, terpenoids, flavonoids, tannins and phenols. For antidiabetic activity, the extract demonstrated potent inhibition of α amylase and α glucosidase enzymes, with IC50 values of 47.12 µg/ml and 49.15 µg/ml, respectively. Regarding antioxidant activity, the extract displayed strong scavenging effect on DPPH free radical, with an IC50 value of 414.228 µg/ml, while standard antioxidant BHT had IC50 value of 50.173 µg/ml, hydrogen peroxide scavenging activity, with an IC50 values 493.656 µg/ml, while ascorbic acid had an IC50 value of 55.789 µg/ml, The extract of lipid peroxidation assay with an IC50 value of 7.786 µg/ml, while BHT had IC50 value 3.198 µg/ml. In conclusion, these findings suggest that the ethanol extract of stem of *Carissa carandas* Linn possesses antidiabetic and antioxidant activities. However further studies are very much needed to explore the mechanism of action and other potential effects using animal studies.

KEYWORD:- *Carissa carandas*; Antidiabetic activity; Antioxidants; Cytotoxicity.

1. INTRODUCTION

Carissa carandas Linn, also referred to as the Karonda christ hawthorn since it grows naturally in bushes. In India's tropical and subtropical Adriatic region, it is only occasionally grown.[1] It is a popular medicinal herb in numerous indigenous medical practises, notably Unani, Ayurveda, and homoeopathy, and is utilised frequently by Indian tribal people (Figure 1).[2] It collects little berries that are widely used to season and preserve Indian pickles and spices. In addition to Karonda karonda (Devanagari: karamardaka (Sanskrit), Koromcha (Bengali), Christ's thorns (South India), vakkay (Telugu), kilaakkaai (Tamil), and Karja tenga (Assamese), the prickly plant is also known by other names.), among others.[3] In present study, evlaution of Anti-diabetic and Antioxidant Potential of stem extract Carissa caranda was done using appropriate methods and guidelines.

Figure 1: *Carissa carandas* linn.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of plant material

Carissa carandas Linn. stem was collected from Birla colony Bhiwani near ITI college of Bhiwani (Haryana) India and authenticated by Dr. S.S Yadav Department of Botany Maharshi Dayanand University, Rohtak (Haryana) India

2.2 Extraction and Phytochemical Evaluation of the Ethanolic extract of *Carissa carandas* Linn. stem (ESCC)

Stems were shade dried, grinded and a coarse powder was obtained which was extracted using methanol as solvent using Soxhlet apparatus. The percentage yield was calculated and quantitative (4) and qualitative (5) phytochemical evaluation was done for the extract.

2.3 Metabolic profiling of Ethanolic extract of *Carissa carandas* Linn. stem (ESCC) by GC-MS analysis

The study involved analyzing the lipid content of Ethanolic extract of *Carissa carandas* Linn. stem (ESCC) using GC-MS. The Methanolic extract was suspended in a methoxylamine hydrochloride solution and GC grade pyridine. The lipid content was analyzed using Thermo Trace GC Ultra coupled with Thermo fisher DSQ II mass spectrometers. Chromatographic separations of metabolites were performed on a 30 m x 0.25 mm Thermo TR50 column. Xcalibur software was used to process the data. The GC oven temperature was maintained at 70°C for 5 minutes, then raised to 290°C. The sample was injected in split mode with helium as a carrier gas. The resulting GC-MS profile was analyzed using Replib, WILLY, and NIST mass spectral libraries.[6] The concentration of metabolites was calculated on the percent peak area basis.

2.4 Evaluation of invitro anti oxidant activity

2.4.1 DPPH scavenging assay

This investigation involves a process of dissolved DPPH in methanol to create a stock solution with a concentration of 0.1 mM. The Ethanolic extract of *Carissa carandas* Linn. stem (ESCC) is then diluted with methanol to create different concentrations. Each test tube receives 3 mL of the stock solution, and the mixture is incubated at room temperature for half an hour. A spectrophotometer is used to test the absorbance at 517 nm, indicating higher antioxidant activity.[7]

2.4.2 Reducing power assay

This investigation involves creating a stock solution of Ethanolic extract of *Carissa carandas* Linn. stem (ESCC) at varying concentrations, preparing dilutions, and combining phosphate buffer, potassium ferricyanide, and the test chemical solution. The reaction mixture is incubated for 20 minutes at 50°C, then centrifuged to remove precipitated proteins. Distilled water and ferric chloride solution are then added to the supernatant. The complex's absorbance at 700 nm is determined using spectrophotometric measurement.[8]

2.4.3 Hydrogen peroxide radical scavenging activity

The assay involved oxidation of potassium iodine (KI) by hydrogen peroxide in iodine ions.[9] The hydrogen peroxide concentration was determined spectrophotometrically using a UV 1800 spectrophotometer. The sample was added to different concentrations of KI and hydrogen peroxide, and the absorbance was measured against a blank solution. Ascorbic acid was used as the standard antioxidant compound.

2.4.4 Lipid peroxidation assay

The lipid peroxidation assay was conducted using the thiobarbituric acid reactive substances (TBARS) method, with slight modifications.[10] A reaction mixture was prepared with flex seed homogenate and different concentrations of sample, and ferrous sulphate solution was added. The reaction was incubated for 30 minutes at 37°C, stopped by adding thiobarbituric acid, heated for 60 minutes at 80°C, and centrifuged for 15 minutes. The absorbance was read against a blank, and the sample OD was calculated by subtracting the per se OD of different test/standards. The experiment was performed in triplicate.

2.5 Evaluation of In vitro Anti-diabetic activities

2.5.1 α-Amylase inhibition activity

The test extract enzyme and soluble starch were dissolved in a 20 Mm sodium phosphate buffer containing 6 ml NaCl. The test extracts were then added to a test tube, pre-incubated at 37°C for 15 minutes, followed by adding 0.5% starch. The mixture was vortexed and incubated again at 37°C for 415 minutes. The reaction was terminated using dinitro salicylic acid color reagent. The tubes were diluted and 200ml of the reaction mixture was placed in a clear plate.[11]

2.5.2 α glycosidase inhibitory essay

The alpha glucosidase inhibitory assay was modified from a previous study.[12] A test extract was prepared in 10 mM potassium phosphate buffer, and a reaction mixture was added to a 96-well clear plate. The mixture was pre-incubated at 37oC for 15 minutes before adding pnitrophenol-a-D-glucopyranoside substrate. After 15 minutes, a stop solution containing 200 mM sodium carbonate was added. The absorbance at 405 nm was recorded using a microplate reader. The inhibition (%) of the test sample on alpha-glucosidase was calculated using the same method as with the alpha-amylase assay. Acarbose, a prescribed drug for alpha-glucosidase inhibition, was used for comparison. The mixture was incubated in triplicate at 37oC for a week.

2.6 In Vitro Cytotoxicity Evaluation of the Compounds-L6 Cell Line by using Neutral red uptake assay

The neutral red uptake assay is a widely used cytotoxicity assay for estimating the number of live cells in a culture. It relies on how well-functioning cells can bind and integrate the supravital dye neutral red in lysosomes. The process involves 96-well tissue culture plates, cell treatment, and incubation in a medium containing neutral red for 2 hours. The absorbance is measured using a spectrophotometer, making it less expensive and more delicate.[13-15]

3. RESULTS AND DISCUSSION

3.1 Percentage (%) Yield and Phytochemical Screening and Quantitative Estimation

The Ethanolic extract of *Carissa carandas* Linn. stem (ESCC) of greenish blue with sticky consistency was obtained with percent yield of 59.06% w/w. ESCC showed presence of carbohydrates, proteins, amino acids, alkaloids, saponins, sterols, tannins and phenolic compounds flavonoids. The total flavonoid content of ESCC was found to be 25.45 ± 0.881 mg quercetin equivalents/g of extract and total phenolic content was found to be 45.08 ± 0.458 mg tannic acid equivalents/g of extract.

3.2 GC-MS Analysis of Ethanolic extract of *Carissa carandas* Linn. stem (ESCC)

The GC-MS chromatogram revealed 12 peaks indicating the presence of various phytochemical components in the ethanolic extracts extracted through Soxhlet extraction. The ethanolic extracts contained active phytochemical components identified by retention time, %area, and molecular formula. The CP-1 (Cyclohexanone) had the least retention time (5.305), followed by CP-2 (Furancarboxaldehyde, 5-methyl), CP-3 (Heptanol, 3-methyl), CP-4 (Phenol, 2-methoxy), CP-5 (Octanoic acid, ethyl ester), CP-6 (Caryophyllene), CP-7 (Diethyl Phthalate), CP-8 (Propyphenazone), CP-9 (Oleic Acid), CP-10 (Octadecanoic Acid), CP-11 (Pentatriacontane), and CP-12 (Stigmasterol).

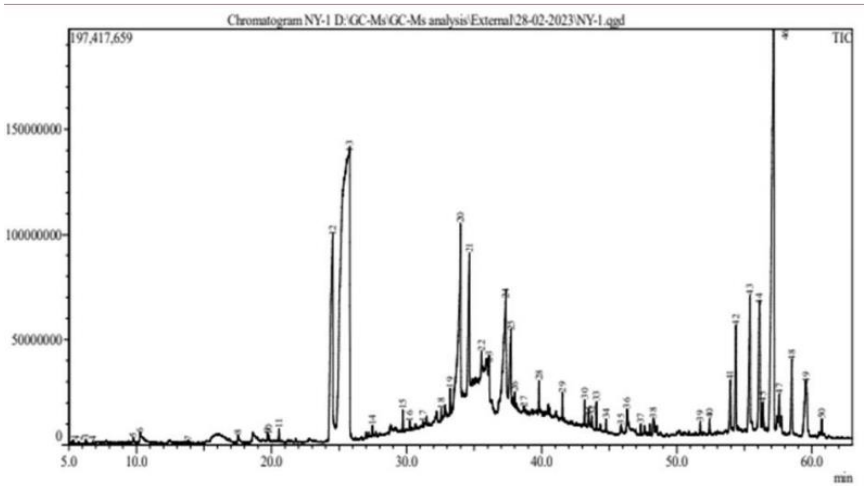

Figure 2: GC-MS Analysis of Ethanolic extract of *Carissa carandas* Linn. stem (ESCC)

3.3 Evaluation of antioxidant activity

3.3.1 DPPH scavenging assay

The study found that ESCC, when combined with standard antioxidant-Butylated Hydroxy Toluene (BHT), effectively inhibited DPPH radical formation in a concentration-dependent manner. The linear regression analysis showed that DPPH scavenging was concentration-dependent, with IC50 values of 414.228, and 50.173 µg/ml, respectively. This suggests that the antioxidants' effectiveness in DPPH scavenging is dependent on their concentration (Table 1).

3.3.2 Reducing power assay

The ESCC in the concentration range of 50-250 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green colour absorbance measured at 700 nm. Similar effect was obtained with standard antioxidant- ascorbic acid in the concentration range of 50-250 µg/ml. A concentration verses absorbance graph comparing ascorbic acid and ESCC were plotted and depicted in Figure 3.

3.3.3 Hydrogen peroxide scavenging activity

ESCC (100-1000 µg/ml) or standard antioxidant ascorbic acid (20-100 µg/ml) showed hydrogen peroxide scavenging as indicated by concentration dependent decrease in the absorbance of H2O2 solution. The linear regression coefficients of ESCC and ascorbic acid were r2 = 0.9155 and r2 = 0.9816, respectively. The IC50 value of ESCC and ascorbic acid were 493.656 and 55.789 µg/ml, respectively (Table 2).

3.3.4 Lipid peroxidation assay

ESCC (2-10 µg/ml) and standard, BHT (1-5 µg/ml) showed anti-lipid peroxidation effect as indicated by concentration-dependent decrease in the absorbance of TBARS showed similar effect. Linear regression analysis of concentration versus percent anti-lipid peroxidation was carried out. The linear regression coefficient of ESCC and BHT were r2 = 0.9844 and r2 = 0.9946, respectively. The IC50 value of ESCC and BHT were 7.786 and 3.198 µg/mL, respectively. The results are expressed in Table 3.

Table 1: DPPH scavenging assay.

Concentration (µg/ml)		% DPPH Inhibition	IC_{50} Value
Sample	100	27.293±0.173	414.228 µg/ml
	200	37.545±0.990	
	400	48.515±0.285	
	600	62.749±0.824	
	800	77.293±0.173	
	1000	87.545±0.990	
BHT	10	13.311±0.397	50.173 µg/ml
	20	25.706±0.529	
	40	47.305±0.496	
	60	65.163±0.636	
	80	75.064±0.223	
	100	80.271±0.257	

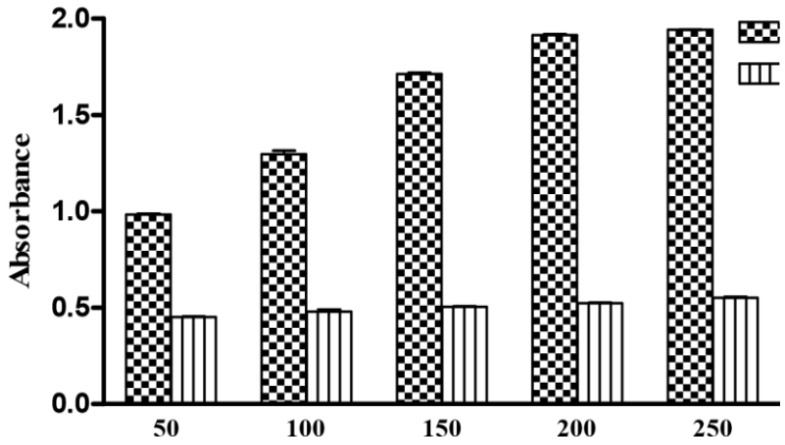

Figure 3: Reducing power assay.

Table 2: Effect of ESCC on hydrogen peroxide scavenging.

Concentration (µg/ml)		% Hydrogen peroxide inhibition	IC_{50} Value
Sample	100	10.569±0.698	493.656 µg/ml
	200	20.467±0.645	
	400	49.062±0.119	
	600	65.685±0.882	
	800	77.281±0.273	
	1000	80.175±0.103	
Ascorbic acid	20	12.179±0.661	55.789 µg/ml
	40	39.364±0.832	
	60	55.504±1.365	
	80	83.470±0.233	
	100	95.614±0.051	

Table 3: Effect of ESCC on lipid peroxidation activity.

Concentration (µg/ml)		% Lipid peroxidation activity	IC_{50} Value
SAMPLE	2	19.424±0.558	7.786 µg/ml
	4	36.462±0.548	
	6	49.148±0.465	
	8	59.799±0.425	
	10	68.820±0.161	
BHT	1	6.650±0.185	3.198 µg/ml
	2	26.780±0.370	
	3	55.929±0.623	
	4	79.428±0.308	
	5	89.057±0.154	

3.4 Evaluation of In vitro Anti-diabetic activities

The ethanolic extract of *Carissa carandas* stem showed the highest inhibition of α-amylase (IC50) compared to acarbose (IC_{50} 40.35 ug/ml) (Table 4; Figure 4). Polyphenols interact with enzymes through non-specific binding, leading to inhibition. Their effectiveness increases with molecular weight and degree of polymerization. The ethanolic extract of *Carissa carandas* Linn. Stem showed the highest inhibition of α-glucosidase, with an IC_{50} value of 49.15 ug/ml (Table 5; Figure 3). Polyphenols interact with the enzyme, increasing their effectiveness with molecular weight and

degree of polymerization. Inhibition of the enzymes α-amylase and α-glucosidase present in the intestine delay the degradation of starch and oligosaccharides to monosaccharides before they can absorb. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose level primarily by interfering with the carbohydratedigesting enzymes and delaying glucose absorption. Thus, inhibition of these enzymes is being related to effectiveness in DM. The stem extracts of *Carissa carandas* Linn ethanolic has shown a significant inhibition of α-glucosidase and α-amylase as compared to standard drug acarbose. It indicates that the stem extracts of *Carissa carandas* Linn potentially act as an antidiabetic.

Table 4: In vitro anti diabetic activity assay (α-Amylase InhibitorAssay).

Concentration (ug/ml)	Acarbose	Sample
200	75.31±1.259	67.80±0.786
100	64.96±1.551	57.26±0.547
50	40.35±0.256	47.12±1.256
25	22.14±1.147	36.16±1.256
12.5	18.22±1.356	25.25±0.658
IC50	40.35 ug/ml	47.12g/ml

Table 5: In vitro antidiabetic assay (α-Glucosidase Inhibitory Assay).

Concentration (µg/ml)	Acarbose	α-Glucosidase Inhibitory Assay
200	75.31 ± 1.259	68.25 ± 0.254
100	64.96 ± 1.551	59.01 ± 1.598
50	40.35 ± 0.256	49.15 ± 1.256
25	22.14 ± 1.147	39.58 ± 0.154
12.5	18.22 ± 1.356	29.56 ± 0.596
IC_{50}	40.35 µg/ml	49.15 µg/ml

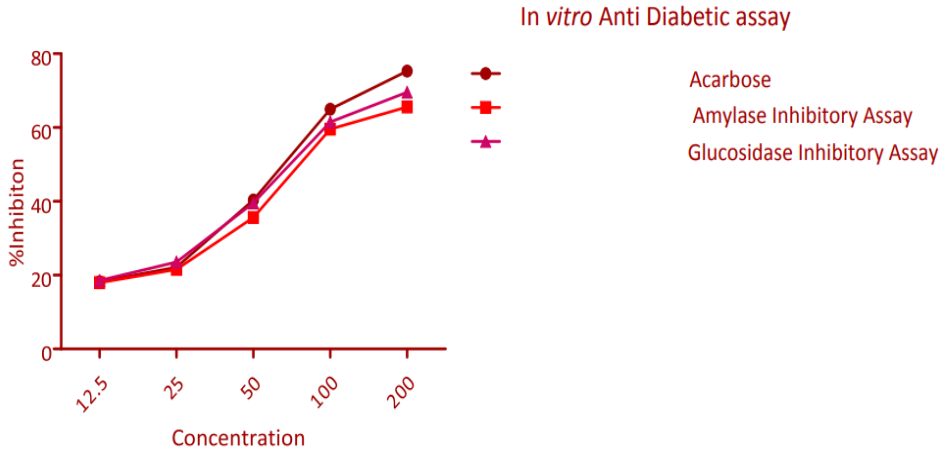

Figure 4: Comparison of In vitro anti diabetic activity of ESCC using αAmylase Inhibitory Assay and α-Glucosidase Inhibitory Assay

3.5 In vitro cytotoxicity Evaluation of the Compound-L6 Cell Line NRU Assay

The cytotoxicity of a ESCC on a L6 cell line was determined using the NRU assay. The cells were cultured in DMEM medium for 24 hours, then fresh culture medium was added, and 5 ul of treatment dilutions were added. The plates were then incubated for 24 hours, then 100 ul of NRU (40 ug/ml in PBS) was added, and incubated for 1 hour. After the medium was removed, NRU was dissolved in 100 ul of NRU destalinisation. The results of the in vitro cell line study are shown in table 6 and figure 5.

Table 6: In vitro cell line study

Sample	IC50 value (ug/ml)
Plant extract	70.97

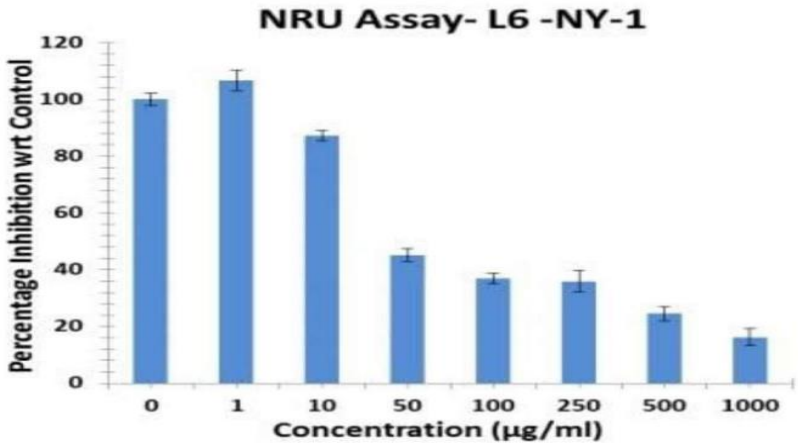

Figure 5: In vitro cell line study.

4. CONCLUSIONS

The study investigated the antidiabetic and antioxidant activities of Carrisa Carandas Linn. using ethanol stem extract. Preliminary studies and GCMS analysis revealed the presence of various phytochemical groups, including carbohydrates, amino acids, alkaloids, terpenoids, flavonoids, tannins, and phenols. The extract showed potent inhibition of α amylase and α glucosidase enzymes, with IC50 values of 47.12 µg/ml and 49.15 µg/ml, respectively. It also showed strong antioxidant activity, with IC50 values of 414.228 µg/ml, BHT of 50.173 µg/ml, hydrogen peroxide scavenging activity of 493.656 µg/ml, and ascorbic acid of 55.789 µg/ml. The ethanol extract of *Carissa carandas* Linn stem exhibited antidiabetic and antioxidant properties. Further studies are needed to explore its mechanism of action and potential effects using animal studies.

5. Conflict of interest

None

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