IJPSR (2022), Volume 13, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 March 2022; received in revised form, 15 May 2022; accepted, 06 June 2022; published 01 November 2022

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF THREE VARIETIES OF *CARISSA CARANDAS* FRUITS AND INHIBITION OF DIGESTIVE ENZYMES RELEVANT TO TYPE 2 DIABETES MELLITUS

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Keywords:

Carissa carandas, Antioxidants, Diabetes mellitus, α-Amylase, α-Glucosidase, Free radicals

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ABSTRACT: *Carissa carandas* is a well-known traditional medicinal plant with a wide range of pharmaceutical properties. In the present studies, three Carissa carandas fruit extract varieties were investigated for the activities relevant to type 2 diabetes mellitus, including inhibition of α-amylase and α-glucosidase. Different solvent extracts such as methanol, ethanol, and aqueous extracts of *Carissa carandas* fruits were evaluated to determine the level of biological activities such as antioxidant activity and free radical scavenging activity by *in-vitro* approaches. Sour green, sour pink and sweet varieties of *Carissa Carandas* fruits were used in this study. Free radical scavenging activity of fruit extracts was assessed using DPPH, H_2O_2, hydroxyl radical, nitric oxide radical, ferric ion, and total antioxidant capacity. Among the three different extracts from the three varieties, the ethanol extract of the sour green variety showed the strongest α- amylase, and α -glucosidase inhibition (IC_{50} = 270.4±1.73 µg/ml and 127.96±0.91 µg/ml), mainly due to the higher antioxidant activity. These extracts were evaluated using various *in-vitro* assays such as hydroxyl radical scavenging activity, nitric oxide scavenging, hydrogen peroxide scavenging, DPPH, and ferrous ion chelating activity. Methanol, ethanol, and aqueous extracts from the three varieties protected the lipids and proteins from oxidative stress. The higher anti-diabetic activity and free radical scavenging activity of sour green fruit ethanol extract can be allocated to a high content of its phenolic compounds. The present study provided data showing the use of green sour fruit extract for therapeutic application.

INTRODUCTION: Diabetes Mellitus (DM) is a chronic metabolic disorder that is characterized by hyperglycemia that occurs either due to the secretion of insufficient insulin or malfunction [1]. Currently, the global diabetes of 537 million adults aged between 20-79 years are living with diabetes and its prevalence is estimated to rise to 783 million by 2045, as a major health concern around the world [2]. The World Health Organization (WHO) shows that India has the largest number of diabetic individuals in the world.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(11).4466-75

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(11).4466-75

The elevated glucose level in the blood following food addiction is one of the major signs in diabetic patients due to the increased breakdown of carbohydrates [3]. The effect of a prolonged rise in blood glucose following a meal is the major complication that leads to morbidity and mortality

among individuals [4]. Disruption in carbohydrates, protein and fat metabolism plays a significant role in the pathogenesis of diabetic complications such as neuropathy, retinopathy, and heart diseases. This triggers the generation of reactive oxygen species (ROS), resulting in cell damage and oxidative stress [5]. Many therapeutic options are available for the treatment of DM with oral synthetic agents and insulin therapy. However, their treatments are commonly associated with disadvantages such as toxicity, drug resistance, relatively high costs, and adverse side effects. As a result, due to its safe and non-toxic nature, the supplementation of medicinal herbal-based treatments to DM is a promising and covered approach [6]. In natural medicine, many plants have been used to treat DM in many countries and have served as useful sources of biological compounds [7]. *Carissa Carandas* (PARC/2022/4685) are commonly available wild plants in India. The fruits, leaves, bark, and roots are used in traditional medicines to treat antibacterial, antipyretic, hepatoprotective, analgesic, and anti-inflammatory disorders [8]. *Carissa Carandas* fruits are rich sources of iron, vitamin C, and antioxidants [9]. Several studies have been reported on the anti-diabetic activities of *Carissa Carandas* fruits [10, 11]. The biological activities of *Carissa Carandas* fruits relevant to antioxidants, radical scavenging activities, and inhibition of key enzymes of starch degradation, including α-amylase and α-glucosidase, are reported in the literature but are limited. No previous studies on different varieties of *Carissa Carandas* fruits from various solvent extracts have been conducted for antioxidant, free radical scavenging, and digestive enzymes relevant to DM. Hence, this is the first study that was carried out to evaluate the effects of aqueous, methanol, and ethanol extracts of three varieties of *Carissa Carandas* fruits such as sour green, sour pink, and sweet variety, on the antioxidant activity, radical scavenging activity, and anti-diabetic activity. The efficacy of the fruit extracts was compared with acarbose, a standard hypoglycemic drug.

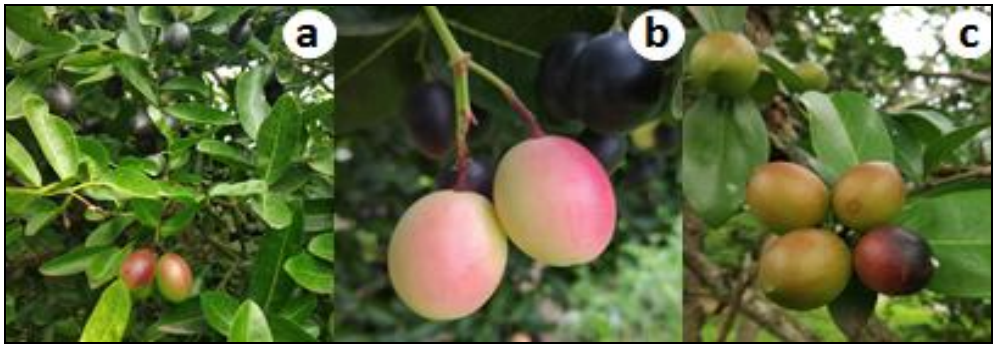


FIG. 1: *CARISSA CARANDAS* FRUIT VARIETIES, A: SOUR GREEN, B: SOUR PINK AND C: SWEET VARIETY

MATERIAL AND METHODS:

Chemicals: The following chemicals were used in this study 2, 2-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide [K3 Fe (CN)], gallic acid (GA), ascorbic acid (AS), and $FeCl_3$, were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA), and ammonium molybdate and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

Preparation of Plant Extracts: The *Carissa Carandas* ripe fruits were harvested and washed thoroughly under running tap water, then oven dried for one week at 40-60 ºC. The dried fruit pulp was uniformly ground using an electric grinder. The powdered material (100g) was extracted for 3 days in 500 mL of distilled water, ethanol, and methanol. The separated extracts were then filtered through Whatman No.1 filter paper, and the filtrates were evaporated to dryness using a rotary evaporator at 40 ºC. The semisolid extract was then dried at room temperature and stored at -20ºC until further use.

2, 2 - diphenyl – 2 - picrylhydrazyl Radical Scavenging Activity: DPPH radical scavenging activity was determined according to the method described by Brand-Williams *et al.* [12]. The dried semisolid extracts were weighed and dissolved in dimethyl sulfoxide (DMSO), and 0.5 mL of each dilution were mixed with 1.5 mL of DPPH solution (0.2mM in methanol) and mixed.

The mixture was kept in the dark at room temperature for 20 min. The absorbance was read at 517 nm in a UV-VIS spectrophotometer, and methanol was used as a blank. Butylated hydroxytoluene (BHT) was used as a positive control, and the experiments were performed in triplicate at each test sample concentration. The percentage inhibition was calculated, and the IC_{50} value was determined.

Hydrogen Peroxide Scavenging Activity: Hydrogen peroxide scavenging activity was measured concentration-dependent; the extract was dissolved in DMSO. The assay mixture contains 1mL of each sample extract and 1mL of H_2O_2 (40mM in 0.05M phosphate buffer, pH 7.4). The mixture was incubated for 15 min and the absorbance was measured at 230 nm in UV-VIS spectrophotometer. Sample extract and buffer without H_2O_2 served as blank and ascorbic acid was used as the experimental control. All assays were performed in triplicate at each concentration of samples. The percentage of H_2O_2 inhibition was calculated from the standard curve obtained from ascorbic acid at different concentrations [13].

Ferrous Ion Chelating Activity: The ferrous ion chelating ability of the fruit extracts was determined by the method described by Chew *et al.* [14] with some modifications. 1.5 mL of different concentrations of the extracts in DMSO was mixed with 0.1 mL of 2 mM $FeCl_2$ solution. The reaction was initiated by 0.4 mL of 2.5mM ferrozine solution. The mixture was shaken well and kept at room temperature for 15 min. After incubation, the absorbance of the assay mixture, which contained the ferrozine-Fe^{2+} complex, was measured at 562 nm by UV-VIS spectrophotometer against a blank which contained DMSO instead of the sample. Ascorbic acid was used as experiment control, and the experiment was performed in triplicate. The percentage of ferrous ion chelating activity was calculated from the standard curve, and the IC_{50} value was determined.

Nitric Oxide Radical Scavenging Activity: Nitric oxide radical scavenging ability of the *Carissa carandas* fruit extracts was determined according to the method described by green *et al.* [15] with some modifications. The different concentration of sample extract (0.5mL) was mixed with 2mL of sodium nitroprusside solution (10mM in 0.05 M potassium phosphate buffer, pH 7.4). BHT was used as standard. The assay mixture was incubated at 25ºC for 3 hrs. From the above solution, 0.5mL was taken and mixed with 0.5mL of griess reagent (1% sulfanilamide and 0.1% naphthlenediamine dichloride dissolved in phosphoric acid). The mixture was further incubated at room temperature for 25 min. The absorbance was read at 540 nm against a blank solution containing a phosphate buffer without a sample extract. All experiments were done in triplicate. The nitric oxide radical scavenging activity percentage was calculated from the standard curve obtained from the BHT.

Hydroxyl Radical Scavenging Activity: The hydroxyl radical scavenging assay was performed by the method of Klein *et al.* [16]. The fruit extracts and ascorbic acid were dissolved separately in DMSO and water. 1 mL of fruit extract with various concentrations was mixed with 1 mL of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.2% EDTA in a ratio of 1:1). After mixing, 0.5mL of 0.02% EDTA solution and 1mL of DMSO were added.

The reaction was initiated by adding ascorbic acid (0.2%) and incubated at 90ºC in a water bath for 20 min. Then, 2mL of 15% TCA was added to the termination of the reaction. 2mL of Nash reagent (ammonium acetate (75g), 3mL of glacial acetic acid, and 2 mL of acetylacetone were mixed, and volume was made up to 1L with distilled water) was added and kept at room temperature for 20 min. The reaction mixture without fruit extract was served as control, and ascorbic acid was used as standard. All the experiments were done in triplicates. A UV-VIS spectrophotometer measured the absorbance of the reaction mixture at 412 nm against a blank solution. The percentage inhibition was calculated from the standard, and the IC_{50} value was determined.

α-Glucosidase Inhibition Assay: The ability of α-glucosidase inhibitory activity of *Carissa Carandas* fruit extracts was determined according to the method given by Kim *et al.* [17] with some modifications. The fruit extracts at various concentrations (100 to 400µg/mL) were dissolved in DMSO, 50 µL of each extract was incubated with 50 µL of 1 Unit/mL α- glucosidase enzyme for

30 min at room temperature. After incubation, the reaction was initiated by the addition of 20µL of 0.5mM pNPG solution, and the reaction mixture was kept at room temperature for 30 min. The reaction was terminated by 1.5 mL of 0.1N Na_2CO_3 solution, and the absorbance of the test sample and blank was read at 405 nm by a UV-VIS spectrophotometer. Acarbose was used as experimental control at various concentrations. The enzyme inhibitory activity was calculated using a standard curve obtained from acarbose, and the concentration required for 50% inhibition of the enzyme activity was determined using a regression equation.

α-Amylase Inhibition Assay: α-amylase inhibitory activity of fruit extract was measured by the method described by McCue and Shetty [18]. The fruit extract, a volume of 250µL at various concentrations, was mixed with 250 µL of 0.1 Units/mL amylase enzymes which was prepared in 0.05M phosphate buffer, pH 6.8 and incubated at room temperature for 10 min. After incubation, 250µL of 1% starch solution was added and incubated at room temperature for 15 min. The reaction was stopped by the addition of 500µL of 1% dinitrosalicylic acid, the tubes were boiled in both water for 10 min. After cooling, the reaction mixture was diluted with distilled water to 10 mL. The absorbance of the sample was measured at 540nm using a UV-VIS spectrophotometer, and acarbose was used as a positive control. The inhibitory activity of the fruit extract was calculated from the standard curve.

Statistical analysis: The experimental results were expressed as mean ± SEM of three replicates. The data were subjected to a one-way analysis of variance (ANOVA) followed by student's *t*-test to calculate the statistically significant differences between the control groups and the test samples. The IC_{50} value was calculated by interpolation from linear regression analysis using XLSTAT software. A value of $p \leq 0.05$ was termed to be significant.

RESULTS AND DISCUSSION:

DPPH Radical Scavenging Activity: DPPH has been used to determine the free radical scavenging capacity of *Carissa carandas* fruit extracts. **Fig. 2** showed the scavenging capacity of the ethanol, methanol, and aqueous extracts were concentration-dependent. In this study, all extracts proved to be effective scavenges of DPPH radical. Ethanol extract of all test samples showed significantly higher inhibition activity, while aqueous extract recorded lower activity. The IC_{50} values were calculated as the concentration of the sample and standard that caused the inhibition of 50% of free radicals.

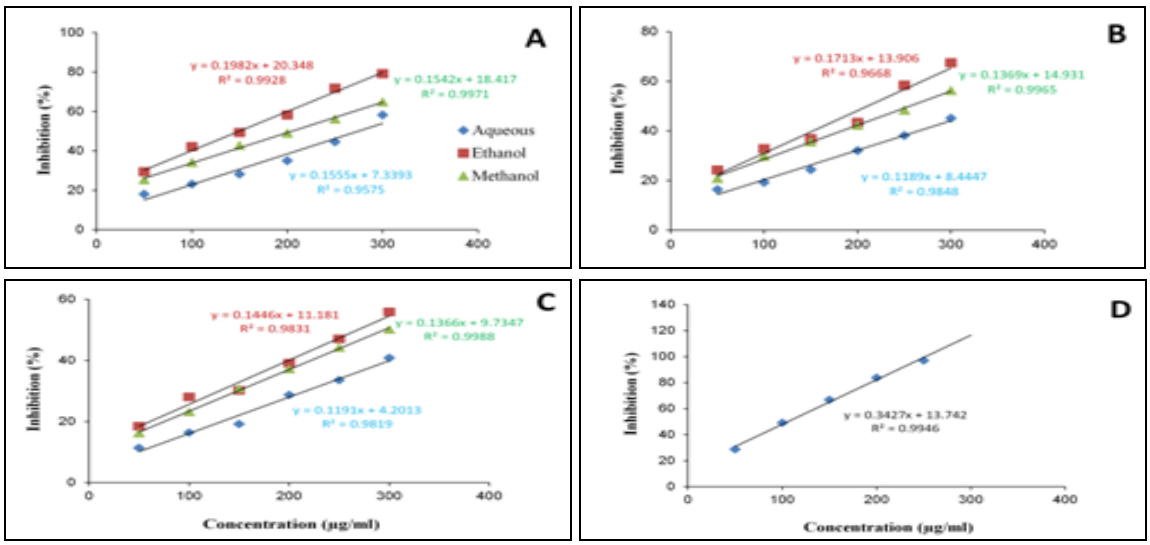


FIG. 2: DPPH RADICAL SCAVENGING ACTIVITY OF *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND BHT (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: BHT).

The IC_{50} values given in **Table 1** of ethanol extract of green sour variety and the standard BHT in this assay were 149.61± 0.87µg/mL and 105.80 ± 0.42 µg/mL, respectively. The IC_{50} values of all extracts

were more than the standard. A lower IC_{50} value indicated a higher scavenging activity. DPPH is a stable free radical used to measure the electron-donating capacity of the molecules, it accepts hydrogen radicals and becomes a stable molecule [19, 20].

The reduction of the DPPH molecule can be achieved by the available hydroxyl groups which are obtained in the antioxidants, including phenolic acids, flavonoids, and anthocyanin [21].

Nitric Oxide Radical Scavenging Activity: The result shown in **Fig. 3** revealed the scavenging activity of the fruit extract against nitric oxide released by sodium nitroprussied. The percentage inhibitory activity of the extracts and standard BHT were compared, and ethanol extract of green sour variety showed significantly higher inhibitory activity (76.12%) compared to other solvent extracts.

An IC_{50} value of the standard (83.72± 0.36 µg/mL) was less than the samples **Table 1**. The inhibition percentage of Green sour methanol and aqueous extracts was recorded at 64.17% and 59.88%, respectively. Lower scavenging activity was observed in the sweet variety in all solvent extracts compared to the sour variety.

TABLE 1: IC_{50} (µG/ML) VALUE FOR DIFFERENT FREE RADICAL SCAVENGING ACTIVITY AND INHIBITION OF CARBOHYDRATE METABOLIZING ENZYMES OF *CARISSA CARANDAS* FRUIT EXTRACTS (A: AQUEOUS; E: ETHANOL AND M: METHANOL)

IC_{50} values	SOUR GREEN			SOUR PINK			SWEET			STD
(µg/ mL)	A	E	M	A	E	M	A	E	M	
DPPH	274.35 ±1.63	149.61 ±0.87	204.85 ±2.01	349.50 ±1.21	210.69 ±1.42	256.17 ±0.86	384.54 ±2.44	268.46 ±1.55	294.77 ±1.03	105.80 ±0.42
Nitric oxide scavenging activity	214.78 ±1.11	147.76 ±0.48	205.65 ±1.47	270.18 ±0.77	207.08 ±1.38	230.80 ±2.05	289.46 ±1.74	241.10 ±0.57	288.18 ±1.89	83.72 ±0.36
H_2O_2 scavenging activity	248.07 ±2.42	135.39 ±0.88	202.29 ±1.65	292.09 ±2.11	198.97 ±0.42	288.88 ±1.22	307.68 ±2.37	272.62 ±1.46	329.34 ±2.01	73.84 ±0.58
Hydroxyl radical scavenging activity	198.67 ±1.08	158.65 ±0.64	176.72 ±1.48	282.69 ±1.53	201.90 ±1.07	225.75 ±1.97	315.04 ±2.14	277.77 ±1.55	273.45 ±1.86	77.83 ±0.77
Ferrous ion chelating activity	218.17 ±1.65	113.91 ±0.59	138.91 ±0.93	273.95 ±1.28	152.70 ±1.77	195.42 ±0.45	288.78 ±2.08	175.36 ±1.27	242.12 ±1.85	87.35 ±0.43
Total antioxidant capacity	264.78 ±0.67	141.18 ±0.88	213.32 ±1.14	310.81 ±1.56	182.26 ±0.43	234.86 ±1.51	347.80 ±1.89	225.45 ±1.22	255.30 ±1.03	57.62 ±0.37
α-Amylase inhibition activity	388.92 ±2.37	270.41 ±1.73	290.06 ±1.29	416.56 ±2.09	318.78 ±0.89	311.02 ±1.54	408.41 ±2.18	340.34 ±1.53	308.75 ±0.97	139.31 ±0.61
α -Glucosidase inhibition activity	200.65 ±1.78	108.20 ±0.52	127.96 ±0.91	219.33 ±1.57	137.22 ±0.73	142.11 ±0.55	236.48 ±1.31	191.82 ±0.75	150.84 ±0.91	156.56 ± 1.02

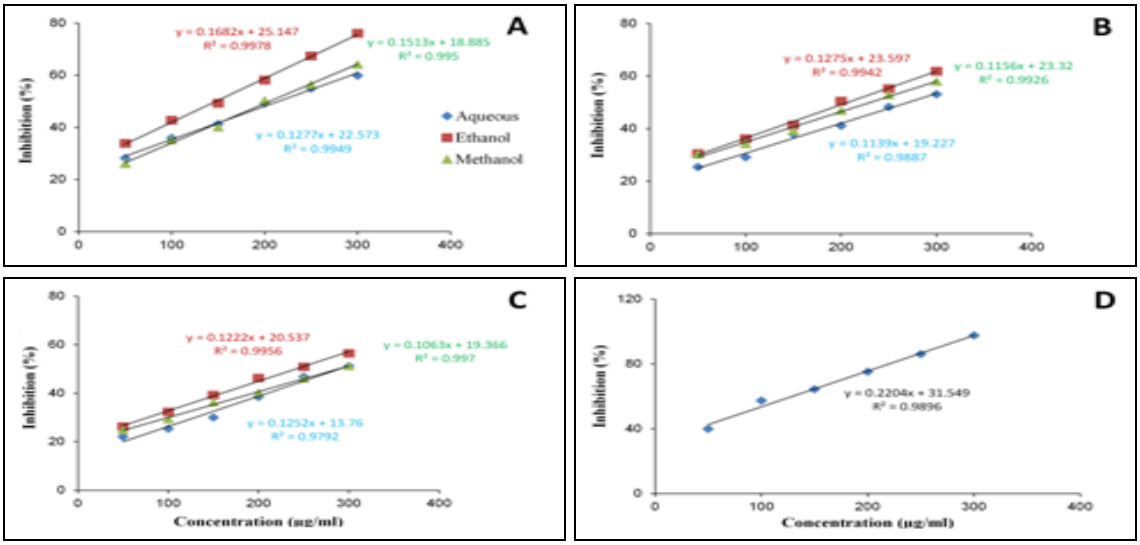


FIG. 3: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND BHT (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: BHT)

Nitric oxide radical is a highly reactive compound generated by endothelial cells, macrophages, neurons, etc. and is responsible for the regulation of various physiological processes [22]. Nitric oxide also reacts with other radicals, including superoxide radicals, to form a peroxynitrite anion that acts as a free radical. The excess of nitric oxide is more toxic to the cells, and leads to oxidative stress-related disorders such as diabetes mellitus, arthritis, cancer, etc. [23]. Our results suggest that more antioxidant principles in the sour green fruit extracts compete with oxygen to react with nitric oxide, thereby preventing the formation of nitrite. Thus, sour green fruit ethanol extracts could be used to manage nitric oxide stress-induced diseases.

Hydrogen Peroxide Scavenging Activity: *Carissa Carandas* fruit extracts were an effective scavenger of hydrogen peroxide radical, and this activity was comparable to that of ascorbic acid (ASA) as a reference compound. **Fig. 4** shows that fruit extracts' hydrogen peroxide scavenging activity was concentration-dependent. The ethanol extract recorded significantly higher scavenging activity than methanol and aqueous extracts of sweet and sour varieties of *C. carandas* fruits. The percentage inhibition activity of green, pink and sweet fruit ethanol extracts were 87.32%, 66.17% and 52.02% with the IC_{50} values 135.38 ± 0.88 µg/mL, 198.96 ± 0.42 µg/mL, and 272.62 ± 1.46 µg/mL respectively **Table 1**. The IC_{50} values of extract were higher than that of the reference compound ascorbic acid (73.84± 0.58 µg/mL). H_2O_2 radical scavenging activity is a useful method for evaluating the antioxidant properties of test samples. H_2O_2 is a weak oxidizing agent. It is further converted to hydroxyl radical in the cell, which may be the more toxic radical that induces cell damage through oxidation of biomolecules [24]. Polyphenols can control the accumulated H2O2 from the cells, which could donate the electrons; thereby, the H_2O_2 is neutralizing into water. The sour green variety showed maximum activity due to its higher concentration of polyphenols.

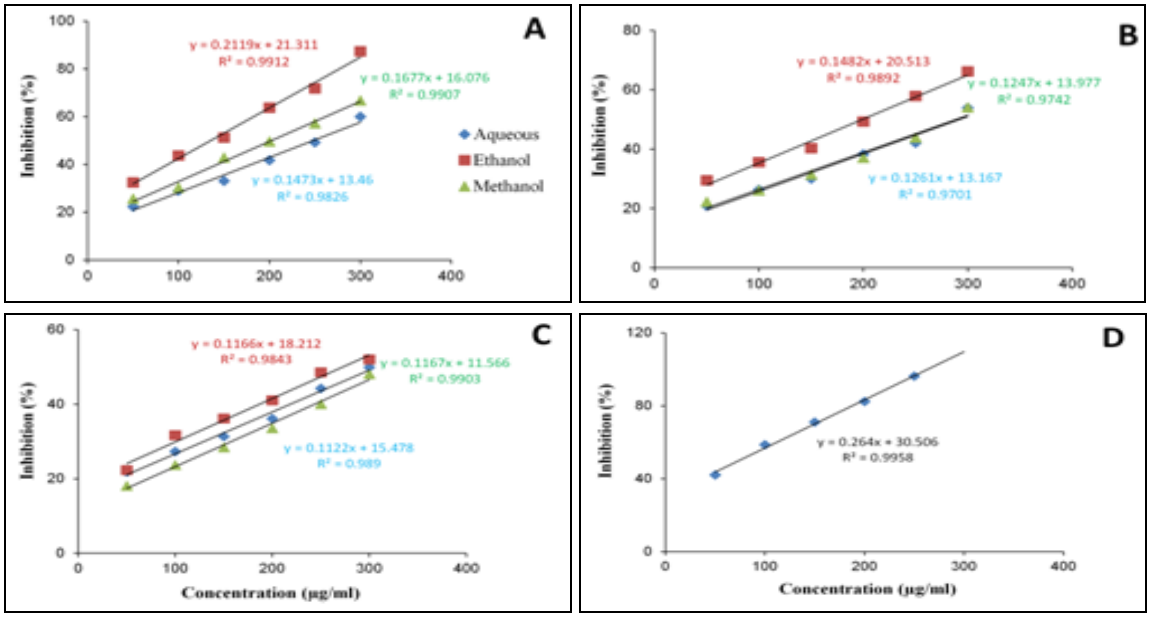


FIG. 4: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY OF THE *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND ASA (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: ASA)

Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging potential of *Carissa Carandas* fruit extract was determined in the concentration range 50-300 µg/mL and the results are presented in **Fig. 5**. A significant activity was observed in ethanol, methanol and aqueous extracts of green fruit compared to pink sour and sweet fruits. The highest percentage (85.73%) of inhibitory activity was recorded in ethanol extract of green fruit, while the lower the activity (49.38%) was observed in aqueous extract of sweet fruit. IC_{50} of the reference compound ascorbic acid was 77.83 ± 0.77µg/mL,

lower than all test samples **Table 1**. Among different solvent extracts and varieties, the green variety recorded lower the IC_{50} value in ethanol (158.64 ± 0.64 µg/mL), methanol (176.72 ± 1.48 µg/mL) and aqueous extracts (198.67± 1.08 µg/mL) respectively **Table 1**. Lower the IC_{50} value indicates a higher antioxidant potential. Hydroxyl radical is an active oxygen species that induce oxidative stress, including lipid peroxidation, protein oxidation, and DNA damage [25]. The present study showed that the sour green fruit ethanol extracts had potent inhibition activity against hydroxyl radicals due to the presence of flavonoids and phenolic compounds in the extracts.

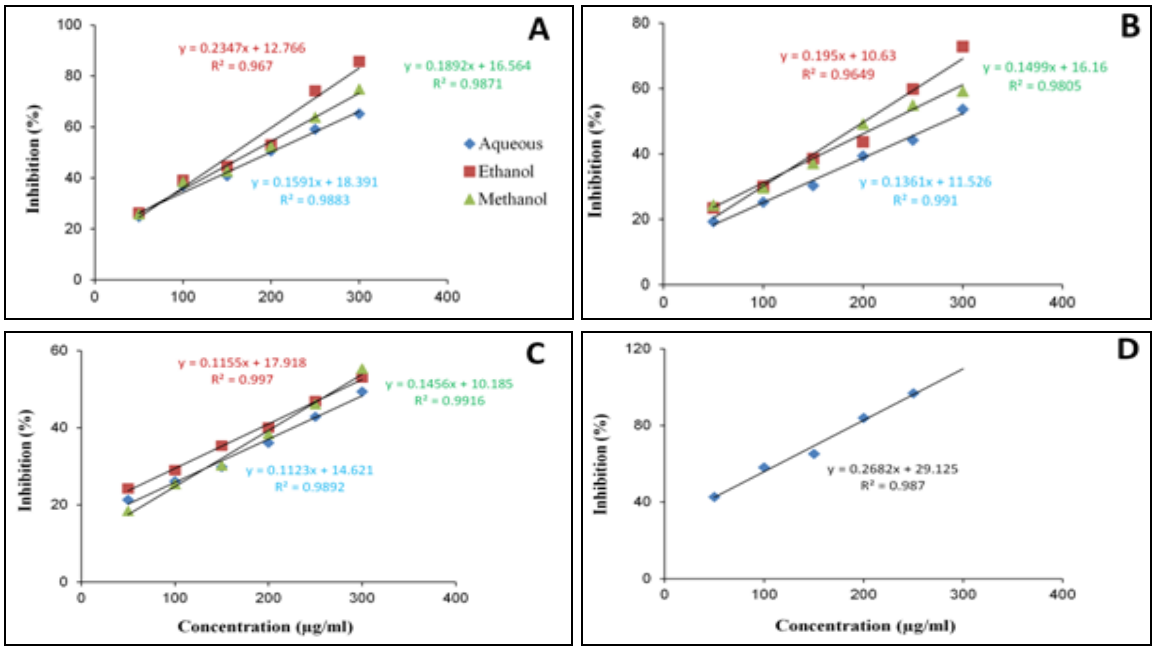
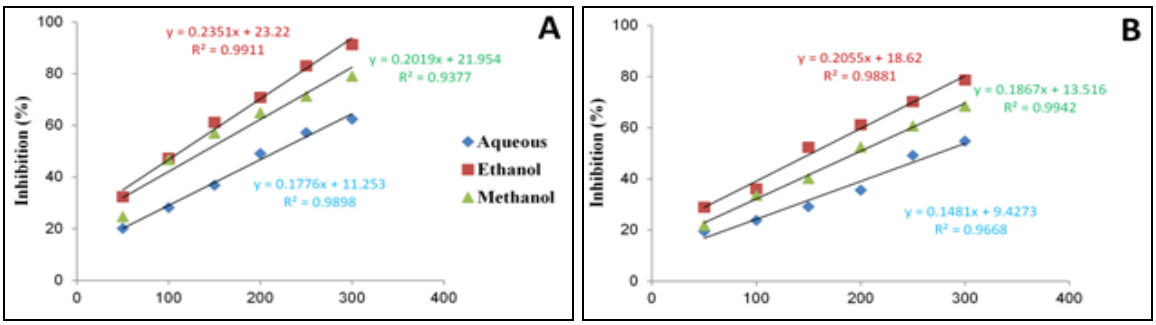


FIG. 5: HYDROXYL RADICAL SCAVENGING ACTIVITY OF THE *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND ASA (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: ASA)

Ferrous ion chelating activity: Fig. 6 shows the dose-dependent ferrous ion chelating activity of *Carissa caranda* fruit extracts compared to that of ascorbic acid as a reference compound. The results indicate that the formation of ferrozine-Fe^{2+} complex is inhibited in the presence of the test sample and the reference compound. The observations demonstrated that the ethanol extract of all samples showed significantly higher chelating activity than methanol and aqueous extracts. An IC_{50} value of ascorbic acid 87.34 ± 0.43 µg/mL is lower than test samples.

The green fruit ethanol extract had lowered (113.91± 0.59 µg/mL) the IC_{50} values than pink (152.71 ± 1.77µg/mL) and sweet (175.36 ± 1.27 µg/mL) variety **Table 1**. The ferrous ion chelating capacity of the plant extracts may serve as an indicator of its potent antioxidant activity [26]. Antioxidant activity is a chain mechanism, including the prevention and decomposition of peroxides. The *Carissa carandas* fruit extracts had ferrous ion chelation activity, increasing with the increasing concentration of these fruit extracts.



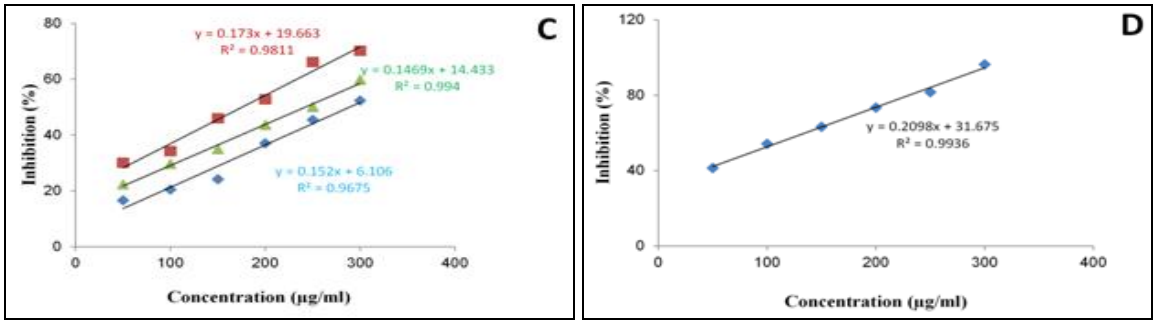


FIG. 6: FERROUS ION CHELATING ACTIVITY OF THE *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND ASA (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: ASA)

Inhibition of α- Amylase and α- glucosidase: Results in Fig. 7 indicate the α-amylase inhibition potential of the *Carissa Carandas* fruit extracts. The inhibition of α- amylase by aqueous, methanol and ethanol extract at 300µg/mL concentration were showed higher inhibition. The inhibitory potential of aqueous extract was significantly different ($P < 0.05$) when compared to other extracts. The results of α- amylase inhibitory activity of fruit extracts was compared with the standard drug acarbose. The calculated IC_{50} value of the extract was 270.41±1.73µg/mL, whereas acarbose was 139.31±0.61µg/mL **Table 1**. All samples' ethanol and methanol extracts recorded the relatively same inhibitory activity. For example, the percentage inhibition of ethanol extract in the pink fruit sample was 48.33%, whereas the methanol extract was 49.35%.

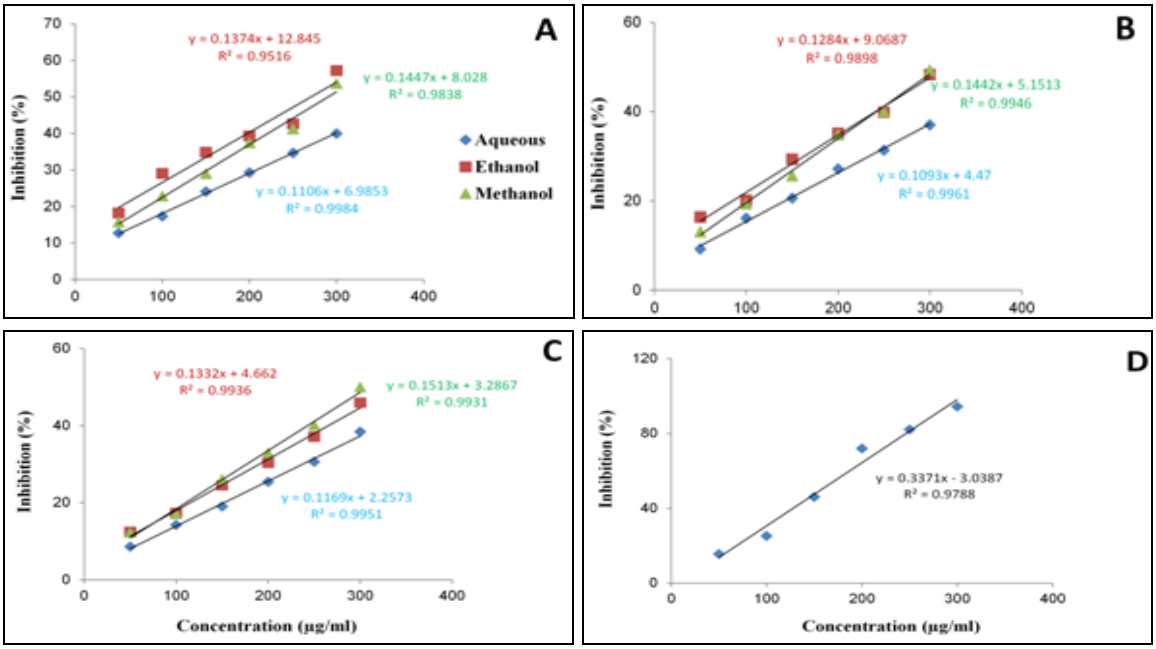


FIG. 7: A- AMYLASE INHIBITION OF THE *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND ACARBOSE (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: ACARBOSE)

Fig. 8 shows the results of the in vitro assay of α-glucosidase inhibitory activity of *Carissa Carandas* fruit extract concentration-dependent. Acarbose was used as a positive control in this study and the IC_{50} value was 156.56±1.02µg/mL **Table 1**. Our results demonstrated that the aqueous extract showed significantly lower inhibitory activity than other extracts. Sour green ethanol extract exhibited maximum enzyme inhibitory activity among the different varieties with 89.31% inhibition (IC_{50} - 108.20±0.52µg/mL), better than the activity observed in the positive control (acarbose), which may support their traditional use as antidiabetic agents. There was no significant difference between ethanol and methanol extract of sour pink variety. The calculated IC_{50} values of extracts

ranged from 108.20±0.52µg/mL to 236.47±1.31µg/mL **Table 1**. Inhibition of amylase and glucosidase delay carbohydrate breakdown in the small intestine and reduce the blood glucose level after the meal in diabetes mellitus [27]. The medicinal plants with greater antioxidant potential and lesser side effects are promising sources for controlling blood sugar levels than existing drugs [28, 29]. The present study showed that the sour green fruit ethanol extract had the greatest amylase and glucosidase activity inhibition. This suggests that the presence of bioactive components in the sour green variety prevents the breaking down of carbohydrates [30]. Our result is in line with previous reports [31] that natural inhibitors of glucosidase and amylase from plants had strong inhibitory activity. Therefore, *Carissa carandas* fruit extracts can be used as potent therapy to reduce postprandial blood glucose levels in diabetes mellitus with no side effects.

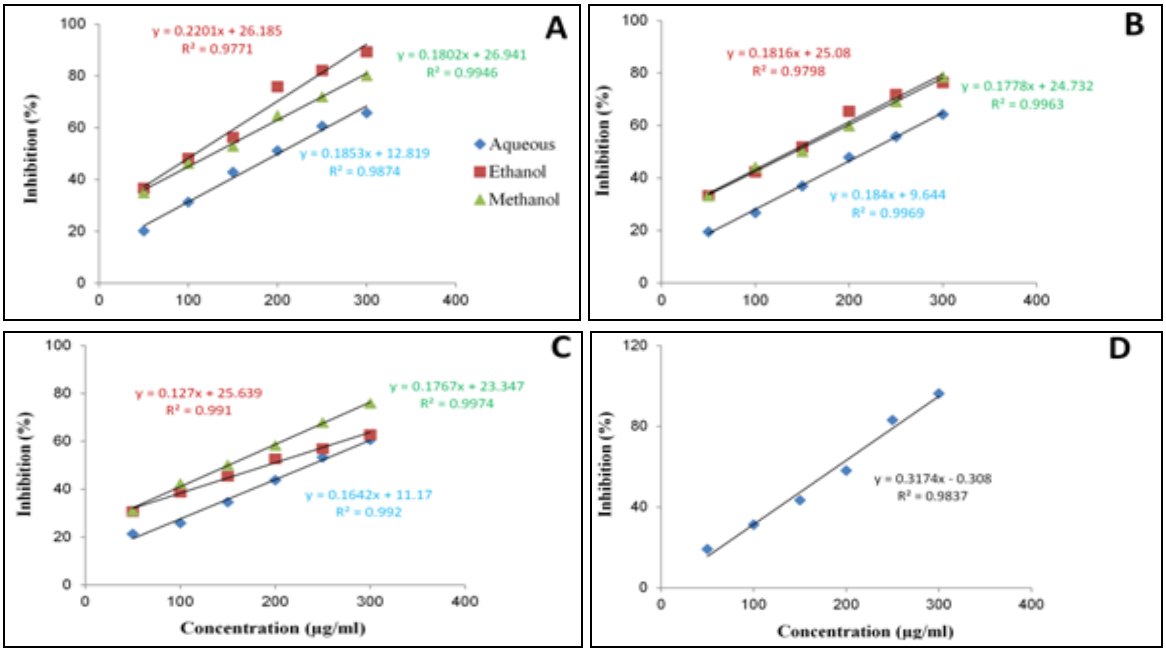


FIG. 8: α- GLUCOSIDASE INHIBITION OF THE *CARISSA CARANDAS* FRUIT VARIETIES IN DIFFERENT SOLVENT EXTRACT AND THE REFERENCE COMPOUND ACARBOSE (PANEL A: SOUR GREEN VARIETY; PANEL B: SOUR PINK VARIETY; PANEL C: SWEET VARIETY AND PANEL D: ACARBOSE)

CONCLUSION: The present study reports inhibitory activities of *Carissa Carandas* fruit extracts against free radicals, α-amylase, and α-glucosidase, with significant inhibition shown in ethanol extracts. The strong inhibition activity of sour green *Carissa Carandas* fruit ethanol extracts indicates that their higher antioxidant potential may be attributed to the presence of polyphenols and other phytochemical constituents in the extracts. The sour green variety of *Carissa Carandas* could be the potential source of natural antioxidant with antidiabetic activity that could have great importance as therapeutic agents in preventing diabetes mellitus and oxidative stress-related disorders.

ACKNOWLEDGEMENT: The authors are thankful to The Secretary and Correspondent, The Principal, Department of Biochemistry, Islamiah Women's Arts and Science College, Vaniyambadi, Tamilnadu, India for providing necessary funding and access to facilities.

CONFLICTS OF INTEREST: The author declares no conflict of interest

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How to cite this article:
Sudha D, Malarkodi R, Gokulakrishnan A and Ali ARL: Antioxidant and free radical scavenging activity of different extracts of three varieties of *Carissa carandas* fruits and inhibition of digestive enzymes relevant to type 2 diabetes mellitus. Int J Pharm Sci & Res 2022; 13(11): 4466-75. doi: 10.13040/IJPSR.0975-8232.13(11).4466-75.