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Original Article

LC-MS/MS AND GC-MS PROFILING AND THE ANTIOXIDANT ACTIVITY OF *CARISSA CARANDAS* LINN. FRUIT EXTRACTS

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ABSTRACT

Objective: The present study was carried out with three varieties (green, pink, and sweet) of *Carissa carandas* fruit extract for the identification of phytochemical constituents in *C. carandas* fruit extracts using Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and Gas Chromatography-Mass Spectrometry (GCMS)

Methods: LC MS/MS and GCMS analysis were adopted to study three varieties of *C. carandas* fruit, namely green, pink, and sweet, using different solvent extractions such as ethanol, methanol, and aqueous.

Results: High levels of phenolic acids and flavonoids in the green variety were beneficial for anti-diabetic activity due to their antioxidant properties. Among the three varieties of tested samples, the maximum concentration was observed in the ethanol extract of the green varieties (2.485 mg/g FW) compared to the ethanol extract of the pink (1.564 mg/g FW) and sweet (1.285 mg/g) varieties, respectively. Ethanol extract of the green variety has a high level of anthocyanin, which increases tolerance to disease. The separation and identification of fatty acids in *C. carandas* fruit were determined through analysis. The sweet *C. carandas* variety recorded the highest concentration of fatty acids (147.2 mg/100g FW) compared to the pink and green varieties (94.9 mg/100 g FW) and (72.79 mg/100 g FW), respectively, and could successfully identify the number of phytonutrients that have health benefits. Further work is being carried out, which may lead to the development of herbal medicine.

Conclusion: The present study concludes that phytochemicals present in *C. carandas* fruit, extracted by LC-MS and GC MS analysis, contain antioxidant and anti-diabetic effects.

Keywords: *Carissa carandas*, Liquid chromatography-mass spectrometry (LC-MS/MS), Gas chromatography–mass spectrometry (GCMS)

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INTRODUCTION

Medicinal plants are important sources of herbal medicines like Ayurvedic, Allopathy, Siddha, Unani, and folk medicine. Traditional medicine is available in the form of powder, paste, infusion, etc., for the treatment of several diseases [1-4]. Most of the plants are naturally producing a widespread amount of biomolecules present in the parts of the plants with medicinal properties that are always developing [5-8].

C. carandas Linn. It belongs to the family F. apocynaceae and is found to be widely used as a medicinal plant by tribals throughout India. The fruit-bearing plant that grows as a tiny shrub is known as karonda [9]. The fruits, leaves, barks, and roots of *C. carandas* have been used for the treatment of many human diseases, such as diarrhea, stomachaches, anorexia, intermittent fever, mouth ulcers, sore throats, syphilitic pain, burning sensations, scabies, epilepsy [10]. *C. carandas* fruit has antioxidant, antidiabetic, antimicrobial, cytotoxicity, anticonvulsant, hepatoprotective, antihyperlipidemic, cardiac depressant, analgesic, anti-inflammatory, antipyretic, antiviral properties, anticancer activity, and lipase conditioning [11, 12]. In *C. carandas* fruits, leaves have been shown to have high antioxidant and DNA damage-inhibiting potential [13]. Chemical constituents include steroids, terpenes, tannins, flavonoids, benzenoids, phenylpropanoids, lignans, sesquiterpenes, and coumarins [14].

However, the bioactive compound responsible for the antioxidant activity of *C. carandas* fruit extracts from different solvent extractions varies among the varieties. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis was used to profile the phenolic, flavonoid, and anthocyanin components. Gas Chromatography-Mass Spectrometry (GC-MS) analysis to assess the fatty acids and organic acids. Additional analysis of this substance leads to the conclusion that *C. carandas* fruits may have medicinal uses.

MATERIALS AND METHODS

Collection of plant materials

The Fruits of *C. carandas* were collected from Jawadhi Hills (Tirupttur, and Thiruvanmalai district, Latitude: 12 34'N Longitude: 78 49'E) as shown in fig. 1, in March 2019. All plant materials and Fruits were identified and authenticated by Prof. P. Jayaraman, a botanist at the Herbarium of Plant Anatomy Research Center. A voucher specimen number PARC/2022/4685 of *C. carandas* has been deposited in the Plant Anatomy Research Center's herbarium, Chennai, Tamil Nadu, India. The fruits were gathered and washed thoroughly under running tap water, then oven-dried for one week at 40-60 °C. The dried fruit pulp was uniformly ground using an electric grinder.

Preparation of the fruit extract

100g of fruit powder was homogenized with 500 ml of water, ethanol, and methanol, filtered through Whatman No. 1 filter paper, and the filter was evaporated to dryness using a rotary evaporator. The fruit extracts of different solvents to LCMS and GCMS analysis. Store the residue at 4 °C for further use [16].

Wash the fruits with water and oven-dry for one week at 40–50 °C. Grind the fruit pulp into powder form, and then 100g of fruit powder was homogenized with 500 ml of water, ethanol, and methanol, filtered through watmann no. 1 filter paper, and the filter was evaporated to dryness using a rotary evaporator. The fruit extracts of different solvents to LCMS and GCMS analysis. Store the residue at 4 °C for further use [16].

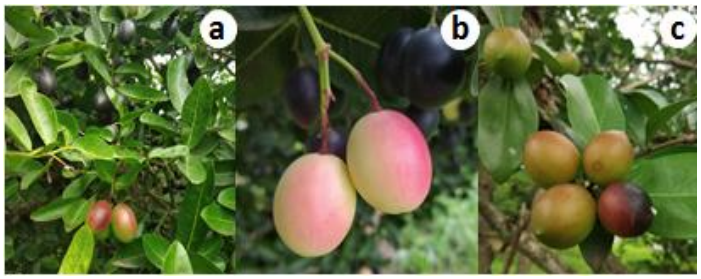

Fig. 1: *C. carandas* fruit varieties, a: green, b: pink, c: sweet variety

Sample preparation

The phenolic acids and flavonoids for LC-MS/MS analysis are isolated from 80% methanol, as described in [17, 18]. Homogenize a 10g fruit in methanol (80%), centrifuge, and make up to 50 ml. Evaporate 20 ml of extract under vacuum at 45 °C and then dilute to 5 ml with water. Extract thrice with petroleum ether in 40 ml of ethyl acetate using a separating funnel. The aqueous layer is essential for extracting phenolic acid, as the ethyl acetate extract evaporates to dryness under vacuum at room temperature. The dry residue was added with 4 ml of 2N NaOH and allowed to hydrolyze overnight. Then, this was acidified to pH 2 using 5 ml of 2N HCl and re-extracted with 50 ml of ethyl acetate. The ethyl acetate layer was again re-extracted twice with 25 ml of 0.1N $NaHCO_3$. The ethyl acetate layer, containing flavonoids, was dried under vacuum. This residue was mixed in 2 ml of MS-grade methanol and filtered through a 0.2µm nylon filter before injection into LCMS/MS for flavonoid estimation. Follow the same steps for the aqueous layer to estimate phenolic acid.

LC and MS/MS conditions

The phenolic acids and flavonoids are resolved in the analytical column BEH-C18 (2.1 x 50 mm, 1.7µm) from Waters India Ltd., protected by a Vanguard BEH C-18 (Waters, USA) with a gradient flow of organic and aqueous phases at a flow rate of 1 ml/min. During analysis, column temperature was maintained at 25 °C, and the sample injection volume was 2 µl. The mobile phase consists of (solvent A) aqueous formic acid (0.1% v/v) and (solvent B) Acetonitrile [18, 19]. A ratio of Solvent 95:5 (A: B) was maintained for 1 min. 5:95 (A: B) for 13-15 min, 95:5 (A: B) for 20 min. The eluted phenolic acids and flavonoids were monitored by a PDA detector at 210-400 nm. Mass spectra of compounds were recorded in the scanning range from M/Z 100-1250. ESI source in both positive and negative ion modes, 600 °Cprobe Temp, 10 ml/min flow rate,45PSI nebulizer gas at 125V. The UPLC column effluent was pumped directly without any split into the TQD-MS/MS (Waters, USA) system optimized for the phenolic acids and flavonoid analysis.

Anthocyanins

Anthocyanins were extracted from the fruit by following the method described [20]. Grind 5g of fruit sample in a mortar pestle using 1% acidified methanol under dark conditions. Using acidic methanol, make up the volume to 50 ml, take 5 ml of the extract from this, dry it under a vacuum evaporator, and dissolve the residue in the mobile phase. Filter it through a 0.2µm nylon filter before injection into the LCMS.

Organic acids

Extraction procedure

Organic acids were extracted as follows, homogenized 5g of sample with 10 ml of 80% methanol, filtered, and dried at reduced pressure [21, 22]. The extract is then allowed to cool and centrifuged at 10000 rpm for 15 min. The supernatant was collected after removing methanol traces using a vacuum evaporator. Filter and extract with 25 ml of ethyl acetate 3–4 times. Remove traces of organic solvent and collect the lower concentrated aqueous phase to reconstitute with water for SPE purification.

Make up the volume with acidified methanol. Dry the elution under nitrogen. Reconstitute (Solvent A and Solvent B; 50:50) in the mobile phase and filter it. 4µL filtrate is injected into the LC-MS/MS for analysis.

LC and MS-MS conditions

The initial gradient is composed of 100% aqueous phase (A) and 0% organic phase (B), held for 0.5 min. At 5.0 min, the gradient is changed to 95% aqueous phase and 5% organic phase, held for 0.5 min, then the system is returned to the initial conditions at 6 min, and this condition is held for 1 min to equilibrate before the next injection. The flow rate is 0.1 ml/min. The analytical column is a 2.1 x 50 mm UPLC BEH-Amide column (Waters) with 1.7µm particles, protected by a Vanguard 2.1 x 5 mm BEH-Amide with 1.7µm particle size guard column (Waters), and the column temperature is maintained at 25 °C. The sample injection volume is 4µl. The eluted organic acids are monitored using a PDA detector, and the UPLC column effluent is pumped directly, without any split, into the TQD-MS/MS (Waters, USA) system, optimized for the identification and quantification of anthocyanin and organic acids.

Fatty acids

GC-FID analysis

GC-FID analysis of fatty acid methyl esters was done using a Varian-3800 gas chromatography system with a flame ionization detector (FID) on a fused silica capillary column (VF-5 Factor, USA), 30 m × 0.25 mm i.d. and 0.25µm film thickness. Helium (99.9%) was used as carrier gas at a constant flow rate of 1 ml/min. The flow rates of H2 and air are maintained at 20 ml/min and 250 ml/min, respectively. The temperature of the column is: initial temperature of the oven at 100 °C for 4 min, then increased by 3 °C per minute up to 220 °C and held for 4 min. Increase the temperature to 260 °C at a rate of 5 °C per minute and hold for 10 min. Injector and detector temperatures are maintained at 250 °C and 260 °C, respectively. Initially, the injection is completed in split-less mode, followed by split mode (1:30) after 1.5 min.

GC-MS

By applying the same temperature program described above for GC-FID analysis, GC-MS analysis was performed on a Varian-3800 gas chromatograph and a Varian 4000 GC-MS ion trap mass selective detector. Helium was accustomed as a carrier gas at a flow rate of 1 ml/min. The temperature is: injector: 260 °C; ion source: 220 °C; trap: 200 °C; transfer line: 260 °C Detector: 260 °C, and injector: 220 °C). The mass scan range was (m/z): 50–450 Atomic Mass Units (amu), Electron Impact (EI): 70. Fatty acids are separated on a VF-5MS fused silica capillary column (Varian, USA) (30 m × 0.25 mm id with 0.25 µm film thickness). Identification of FA was completed by comparing the relative retention times of FAME peaks with a reference standard (Sigma-Aldrich, USA). Spectral data can be found in the Wiley and NIST-2007 libraries [23].

RESULTS

Table 1. Shows the results of *C. carandas* fruit sample varieties (green, pink, sweet) extracted by ethanol, methanol, and water. The green fruits had a higher concentration of phenolic acids (1889.15 mg/100g) compared to sweet (1249.82 mg/100g) and pink

(1507.43 mg/100g) varieties done by LCMS. The green variety had higher concentrations of ferulic acid, gallic acid, 2,4-dihydroxy benzoic acid, t-cinnamic acid, vanillic acid, and salicylic acid when compared to the sweet and pink varieties. The ethanol extract of *C. carandas* fruits contains higher concentrations of phenolic acids when compared to methanol and aqueous extracts.

Table 1: Profiling of phenolic acids in different extracts of green, pink, and sweet varieties of *Carissa carandas* fruits

Phenolic acids	Green			Pink			Sweet		
(mg/100g FW)	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
p-Hydroxybenzoic acid	25.55	30.1	28.88	9.34	23.44	20.14	7.41	18.32	11.78
Salicylic acid	20.37	40.6	35.35	15.47	32.14	23.47	12.02	28.47	20.48
2,4-Dihydroxy benzoic acid	0.22	0.75	0.49	0.37	0.57	0.22	0.25	0.63	0.41
Gentisic acid	2.52	42.77	25.34	1.34	33.22	18.41	1.02	30.28	22.37
Protocatechuic acid	0.63	2.38	1.7	0.44	2.04	2.3	0.18	2.77	1.22
p-Coumaric acid	15.89	64.89	62.58	8.14	53.79	49.11	7.11	46.02	35.88
o-Coumaric acid	4.9	8.12	12.74	2.37	9.37	8.97	2.04	7.11	7.04
Vanillic acid	33.11	431.06	182.49	19.78	288.14	161.22	13.79	247.04	124.08
Gallic acid	1.96	33.04	6.51	1.1	25.84	5.4	0.79	16.28	3.7
Caffeic acid	0.17	0.78	1.96	0.22	0.97	2.1	0.34	0.34	1.7
Ferulic acid	230.72	920.01	411.32	198.25	788.12	353.44	127.18	657.19	292.43
Syringic acid	109.91	124.01	138.78	88.47	105.3	117.11	92.47	84.07	125.01
Chlorogenic acid	0.23	0.38	0.85	0.11	0.19	0.58	0.34	0.27	0.33
t-Cinnamic acid	94.08	190.26	104.09	78.44	144.30	88.22	53.49	111.03	55.77
Total	540.26	1889.15	1013.08	423.84	1507.43	850.69	318.43	1249.82	702.2

The MRM details of phenolic acid analysis are depicted in table 2.

Table 2: MRM details for phenolic acids

Compound	Formula/mass	Parent m/z	Cone voltage	Daughters	Collision energy	Ion mode
Caffeic acid	180	178.90	30	135.05	16	ES-
2,4-Dihydroxybenzoic acid	154	152.90	28	65.02	18	ES-
Chlorogenic acid	354	352.97	22	191.10	18	ES-
Ferulic acid	194	192.90	26	134.02	14	ES-
Gallic acid	170	168.90	28	125.03	12	ES-
Gentisic acid	154	152.90	24	108.98	12	ES-
o-Coumaric acid	164	162.90	22	119.06	12	ES-
p-Coumaric acid	164	162.90	24	119.05	14	ES-
p-Hydroxybenzoic acid	138	136.90	26	93.01	12	ES-
Protocatechuic acid	154	152.90	26	109.05	16	ES-
Salicylic acid	138	136.90	28	93.10	14	ES-
Syringic acid	198	196.97	26	182.07	10	ES-
t-Cinnamic acid	148	146.90	26	103.05	10	ES-
Vanillic acid	168	166.97	26	108.01	20	ES-

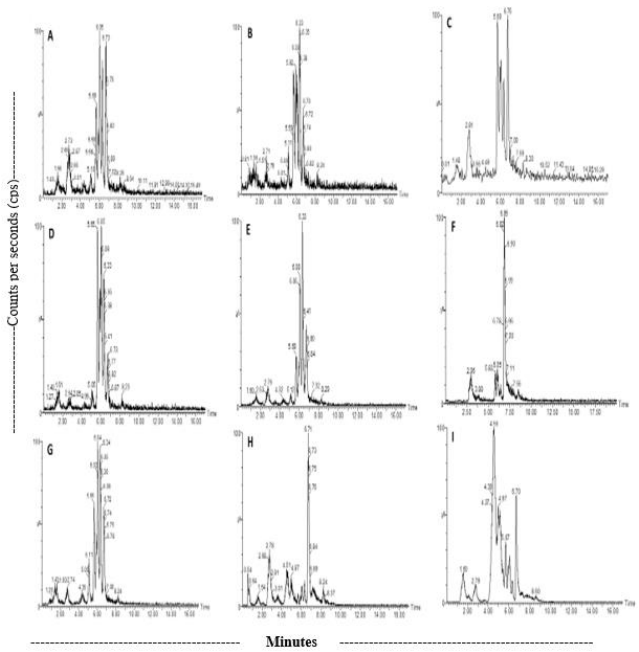

Fig. 2: Chromatogram details of phenolic acids present in *C. carandas* fruit extract analyzed by LCMS. A, B and C indicates samples of sour green aqueous, methanol and ethanol extracts. D, E and F showed the results of sour pink aqueous, methanol and ethanol extracts. G, H and I indicate the sweet variety aqueous, methanol and ethanol extracts

Table 3: Profiling of flavonoids in different extracts of green, pink, and sweet varieties of *C. caranda* fruits

Flavonoids	Green			Pink			Sweet		
(µg/100g FW)	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Umbelliferone	156.22	321.75	292.08	119.44	286.51	231.77	84.25	235.77	227.84
Apigenin	7.34	12.87	13.47	3.78	19.87	8.36	2.14	15.67	9.32
Neringenin	33.12	117.44	102.75	19.12	53.87	48.11	23.07	27.18	23.42
Luteoline	21.7	34.14	28.79	14.79	21.69	22.73	8.99	22.78	16.83
Catechin	125.43	204.32	179.49	109.24	132.87	124.33	79.39	108.57	87.39
Hesperitin	43.01	64.28	55.22	38.79	56.33	38.21	41.02	27.85	17.05
Quercetin	113.07	155.81	97.15	127.02	135.1	72.41	114.08	73.42	62.79
Myrcetin	1204.42	2845.57	2732.11	908.79	1814.08	1675.23	795.28	987.49	1124.37
Rutin	5125.44	9264.24	8997.37	3734.28	7578.43	6784.34	3897.11	5358.65	4879.34
Kaempferol	108.03	142.15	136.47	111.78	278.34	188.58	124.33	172.55	143.18
Epicatechin	102.52	138.26	133.89	66.49	88.39	75.29	48.97	102.74	97.49
Epigallo catechin	38.27	86.52	92.49	25.71	34.78	48.47	18.51	51.87	36.23
Total	7078.57	13387.35	12861.28	5279.23	10500.26	9317.83	5237.14	7184.54	6725.25

Table 4 displays the result of MRM details of different flavonoid compounds. Fig. 3. Represent the chromatogram details of flavonoids present in the fruit extract analyzed by LCMS.

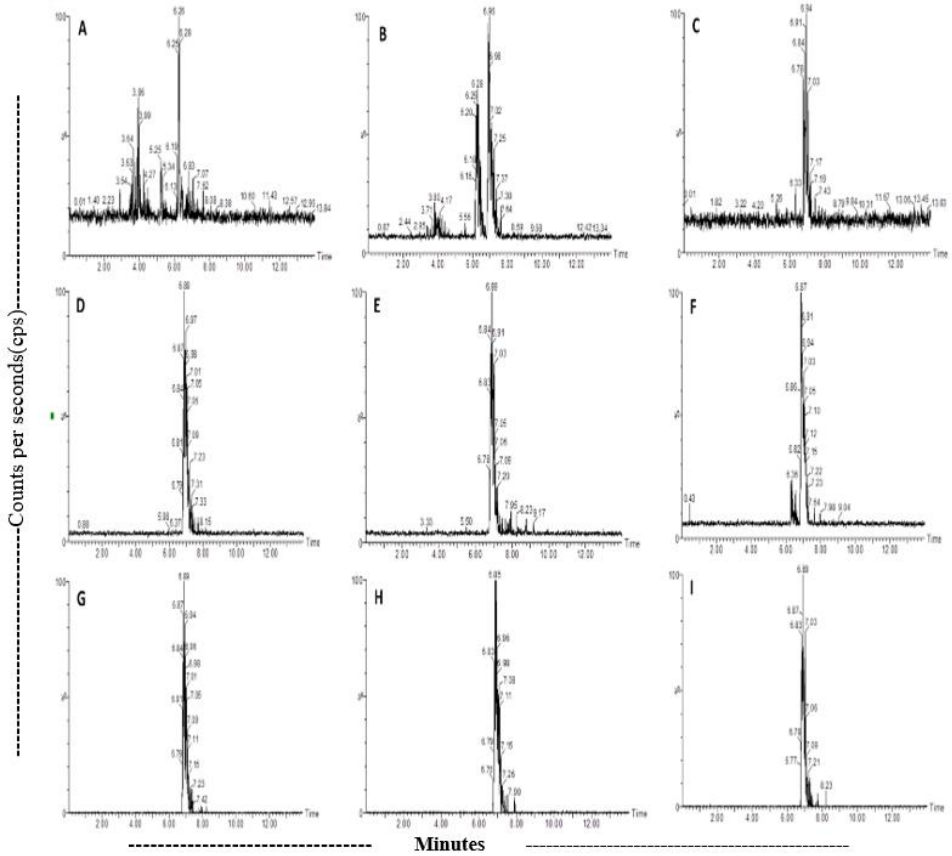

Fig. 3: Chromatogram details of flavonoids present in *C. carandas* fruit extract analyzed by LC-MS. A, B and C indicates samples of sour green aqueous, methanol and ethanol extracts. D, E and F showed the results of sour pink aqueous, methanol and ethanol extracts. G, H and I indicate the sweet variety aqueous, methanol and ethanol extracts

Table 4: MRM details for flavonoids

Compound	Formula/Mass	Parent m/z	Cone voltage	Daughters	Collision energy	Ion mode
Apigenin	270	268.97	46	107.04	30	ES-
Catechin	290	289.03	38	245.05	12	ES-
Hesperetin	302	300.97	42	286.15	16	ES-
Kampherol	286	284.97	54	145.5	36	ES-
Quercetin	302	301.03	36	151.12	20	ES-
Rutin	610	609.1	60	300.2	42	ES-
Epicatechin	290.27	289.05	20	245.15	15	ES-
Epigallo catechin	306.27	305.05	20	219.05	15	ES-

Table 5: Profiling of anthocyanins in the different extract of green, pink and sweet variety of *C. caranda* fruits

Anthocyanins	Green			Pink			Sweet		
(mg/g FW)	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Cyanidin-3-glucoside	0.78	1.88	1.74	0.35	1.31	1.48	0.24	1.09	1.12
Delphinidin-3-glucoside	0. 31	0.441	0.37	0.09	0.157	0.11	0.063	0.104	0.12
Pelargonidin-3-glucoside	0.031	0.073	0.056	0.024	0.040	0.037	0.021	0.052	0.029
Malvidin 3-glucoside	0.028	0.091	0.083	0.038	0.057	0.065	0.018	0.039	0.025
Total	0.059	2.485	2.249	0.502	1.564	1.692	0.342	1.285	1.294

Table 5 shows the anthocyanins present in the *C. carandas* sample extracts. The presence of anthocyanins in different extracts, including cyanidin 3-glucoside, delphinidin 3-glucoside, malvidin 3-glucoside, and malvidin 3-glucoside, was identified by LCMS analysis. Among the tested samples, the maximum concentration was observed in the ethanol extract of the green variety (2.485 mg/g FW) compared to the ethanol extract of the pink variety (1.564 mg/g FW) and the sweet variety (1.285 mg/g FW), respectively.

The organic acid profile in *C. carandas* fruit samples was done with different solvent extracts (table 6). The fruit samples revealed the presence of organic acids like oxalic acid, maleic acid, citric acid, tartaric acid, malic acid, ascorbic acid, shikimic acid, and fumaric acid. Among these, malic acid was the major component in all samples. The aqueous extract of all samples recorded the highest concentration of organic acids compared to the ethanol and methanol extracts. The aqueous extract of the green variety showed higher levels (3045.86 mg/100 g FW) compared to the pink (2665.07 mg/100g FW) and sweet (1227.55 mg/100g FW) varieties. Evidence suggests that organic acids such as malic or citric acids may have a positive health benefit as antioxidants.

Table 6: Profiling of organic acids in different extracts of green, pink, and sweet varieties of *C. caranda* fruits

Organic acids	Green			Pink			Sweet		
(mg/100g FW)	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Oxalic acid	2.97	2.45	1.18	2.14	1.87	0.94	2.56	2.22	0.87
Maleic acid	0.55	0.39	0.19	0.37	0.25	0.13	0.3	0.24	0.12
Citric acid	345.03	308.21	189.79	227.51	203.14	112.32	162.65	143.57	69.55
Tartaric acid	16.04	13.87	8.97	10.26	8.97	5.89	14.2	12.39	4.87
Malic acid	2612.43	1874.13	745.27	2373.07	1758.25	677.84	1003.1	945.87	389.72
Ascorbic acid	61.19	45.98	28.77	47.73	33.97	22.98	43.05	36.91	24.22
Shikimic acid	6.94	5.32	1.48	3.71	3.24	1.85	1.34	1.08	0.37
Fumeric acid	0.71	0.48	0.087	0.28	0.15	0.079	0.35	0.21	0.08
Total	3045.86	2250.83	975.737	2665.07	2009.84	822.029	1227.55	1142.49	489.8

The essential fatty acid profiling in all the sample extracts was analyzed by GCMS. The result is presented in table 7.

Table 7: Profiling of fatty acids in green, pink and sweet varieties of *C. carandas* fruits

Profiling	Fatty acids (mg/100g FW)	Green	Pink	Sweet
C8:0	Caprylic	0.055	0.043	0.074
C9:0	Nonanoic	0.014	0.027	0.043
C10:0	Capric	0.380	0.090	0.170
C11:0	Undecanoic	0.057	0.145	0.217
C12:0	Lauric	0.017	0.015	0.065
C13:0	Tridecanoic	0.381	0.742	1.160
C14:0	Myristic	0.039	0.075	0.152
C15:0	Pentadecanoic	0.652	1.290	1.174
C16:0	Palmitic	33.871	37.801	52.841
C16:1	Palmitoleic	0.336	0.588	1.385
C17:0	Heptadecanoic	8.282	11.173	26.931
C18:0	Stearic	13.745	10.910	9.000
C18:1	Oleic	6.656	24.921	34.351
C18:2	Linoleic	1.655	1.154	2.214
C18:3	Linolenic	0.035	0.321	0.123
C19:0	Nonadecanoic	0.017	0.023	0.088
C20:0	Arachidic	0.125	0.252	0.612
C20:1	Eicosenoic	0.041	0.175	0.246
C21:0	Heneicosenoic	0.076	0.176	0.082
C22:0	Behenic	1.658	0.016	1.967
C22:1	Erucic	0.285	0.492	2.326
C23:0	Trieicosenoic	0.360	0.382	1.143
C24:0	Tetraeicosenoic	0.739	1.098	3.466
C26:0	Hexaeicosenoic	2.928	2.255	5.654
C28:0	Octaeicosenoic	0.391	0.782	1.722
	Total	72.795	94.946	147.206

The sweet *C. carandas* variety recorded the highest concentration of fatty acids (147.2 mg/100g FW) compared to the sour varieties of pink and green (94.9 mg/100 g FW) and 72.79 mg/100 g FW, respectively. Oleic acid was the main mono-unsaturated fatty acid

present at the highest concentration in the sweet variety (34.35 mg/100g FW) compared to the sour varieties (24.92 mg/100g FW in pink and 6.66 mg/100g FW in green), respectively. The main component observed was the saturated fatty acid (SFA) palmitic acid, which had a significantly higher concentration (52.84 mg/100g FW) in the sweet variety compared to the sour variety. Essential fatty acids are important to cells and good health; they must be consumed via diet, as the human body cannot synthesize them. The key lipid units present in fatty acids are necessary for human nutrition as a source of energy and for physiological and structural functions.

DISCUSSION

The present study found that *C. carandas* fruits of green, pink, and sweet varieties were extracted in different solvents. The green variety showed the highest phytochemical content. The ethanol extract from the green variety had a higher concentration of phenolic acids when compared to the sweet and pink varieties. The green variety had higher concentrations of ferulic acid, gallic acid, 2, 4-dihydroxybenzoic acid, t-cinnamic acid, vanillic acid, and salicylic acid when compared with methanol and aqueous extracts. Reports suggest that many phytochemicals from plants were extracted by ethanol [24]. The results of various phenolic compounds in *C. carandas*, such as vanillic acid, ellagic acid, and gallic acid [25, 26] are similar to the present study. Higher phenolic acids in green fruits demonstrate that ethanolic extract is a potent source of novel bioactive compounds and an alternative for managing oxidative stress-induced diabetes.

Ethanol extracts of the green variety show higher concentrations of flavonoids (13387.35 µg/100g) compared to methanol (12861.28 µg/100g) and aqueous (7078.57 µg/100g) extracts. Flavonoids, known as scavengers, strongly eliminate oxidative radicals. Rutin is the principal flavonoid compound in C. carandas fruits. The high concentration of flavonoids in ethanolic extract may account for the highest antioxidant activity of the green fruits in our study. Flavonoids are most widely present in plants, such as quercetin, catechin, and kaempferol. The radical-scavenging activity of a flavonoid compound may depend on the number of hydroxyl groups present in the molecule. Quercetin was found to protect DNA damage in human lymphocytes induced by hydrogen peroxide. This protection might be due to the dihydroxy structure of both flavonoids being essential for the inhibition of DNA damage [27-29], which demonstrated that, in addition to Quercetin, kaempferol could also inhibit H_2O_2-induced DNA strand breaks in human lymphocytes. The quercetin decreased lipid peroxidation and protein oxidation against dimethoate-induced oxidative stress by increasing superoxide dismutase and catalase enzyme activities in human lymphocytes [30]. Ethanol extract of green fruit in our study may increase antioxidant enzyme activities to protect DNA from oxidative stress. Anthocyanin contents in different solvent extracts of *C. caranda* fruit varieties were observed to be varied in each extract. Among the tested samples, the maximum concentration was present in the ethanol extract of the green variety (2.485 mg/g FW) compared to the pink variety (1.564 mg/g FW) and the sweet variety (1.285 mg/g FW), respectively. Aqueous extracts of all the test samples recorded a lower concentration of anthocyanins when compared to ethanol and methanol extracts. Cyanidin-3-glucoside was the major anthocyanin in *C. carandas* fruit extract. Anthocyanin plays a significant role in antioxidant activity against free radical-induced oxidative stress. The conjugated structure of anthocyanin evaluated maximum antioxidant activity because of its better electron delocalization ability [31]. The green variety with a higher anthocyanin content may act as a free radical scavenger. The results were similar to those of other studies where different berries were evaluated for their antioxidant activity [32-34]. Organic acids like oxalic acid, maleic acid, citric acid, tartaric acid, malic acid, ascorbic acid, shikimic acid, and fumaric acid are present in the examined fruit. The aqueous extract of the green variety showed higher levels of malic and citric acid when compared with the pink and sweet varieties of *C. carandas* [35, 36]. Evidence suggests that organic acids such as malic or citric acids act as antioxidants and esters, with an increasing number of commercial applications as organic compounds [37]. The fatty acid constituents of *C. carandas* was evaluated by GCMS. The sweet variety shows the highest concentration of fatty acids (147.2 mg/100g FW) when compared with the pink and green varieties (94.9 mg/100 g FW, 72.79 mg/100 g FW, respectively). The sweet variety contains palmitic acid (saturated fatty acid), Oleic acid (mono-unsaturated fatty acid) [38, 39], and heptadecanoic acid (unsaturated fatty acid) in higher concentrations when compared with other varieties. Essential fatty acids are important to cell structure and good health.

CONCLUSION

The present study found that *C. carandas* fruits of green, pink, and sweet varieties were extracted in different solvents. The green variety showed the highest phytochemical content with LC-MS profiling when compared with other varieties. Ethanol extract of all varieties showed the highest phenolic and flavonoid content. The Anthocyanin content of the green variety was several times higher than other varieties, which makes it appropriate for preventing lifestyle diseases. Aqueous extract of green variety showed higher levels of organic acids. Sweet variety shows the presence of elevated levels of fatty acids by GCMS analysis. The differences in phytochemical value among *C. carandas* varieties have a great role as an antidiabetic fruit.

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ABBREVIATION

LCMS/MS-Liquid chromatography-mass spectrometry, GCMS-Gas chromatography-Mass spectrometry, FW-Fruit weight, DNA-Deoxyribonucleic acid, NaOH-Sodium hydroxide, $NaHCO_3$-Sodium bicarbonate, PDA–Photodiode array, GC-FID-Gas chromatography Flame ionization detector

AUTHORS CONTRIBUTIONS

Sudha completed the research work plan and Manuscript writing. Malarkodi did the review of the literature collection, Gokulakrishnan did the work plan and Manuscript corrections. All authors have read and agree to the manuscript's published version.

CONFLICTS OF INTERESTS

The authors declared no conflicts of interest.

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