

Identification Of Bioactive Phytochemicals By GC-MS Profiling, Phytochemical Screening, And TLC Using Methanolic Extract Of *Carissa Carandas* Linn Leaf

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Abstract

Background

Carissa carandas (Apocynaceae) is a thorny evergreen shrub known as Karonda. It has small berry-shaped fruits, used as an additive in many pickles or spices in northern India.

Objective

The objective was to identify the phytochemical constituents, gas chromatography-mass spectrometry (GC-MS), and TLC screening by using a methanolic extract from the leaves of *Carissa carandas*.

Methods and materials

Leaves of *C. carandas* were collected, shade dried, pulverized, and extracted successively with methanol by the Soxhlet technique. The crude extracts were later exposed to phytochemical screening for secondary metabolites, thin-layer chromatography, and GC-MS analysis.

Result

The phytochemical screening revealed the presence of alkaloids, saponin, tannin, flavonoid, phenol, steroid, carbohydrate, glycosides, and terpenoids. TLC analysis was performed, and the R_f value was calculated. The GC-MS analysis determined the presence of 6 main phytochemical compounds in the methanolic extract of *Carissa carandas* leaf, showing that there were different types of high and low molecular weight compounds.

Conclusion

The results of the present study pave the invention of herbal medicines for several ailments by using the *Carissa carandas* plant, which may lead to the development of novel drugs.

Keywords: *Carissa carandas*, Apocynaceae, GC-MS, TLC, Phytochemical screening, antioxidant

1. INTRODUCTION

Carissa carandas Linn is a fruit-bearing plant that develops into a small shrub in the Apocynaceae family. It has long been utilized as an Ayurveda, Unani, and homeopathic medication and is extensively spread in subtropical and tropical areas (1). *C. carandas* has been regarded in numerous traditional medical systems as a treatment for various ailments (2). The unripe fruit is thermogenic, aphrodisiac, appetizer, and antipyretic and is beneficial for Pitta and Kapha imbalances, hyperplasia, diarrhea, anorexia, and intermittent fevers (3). The ripe fruit is appetizing and antiscorbutic, and it will be used to treat burning sensations, skin illnesses, scabies, and itching (4). Compared to other tropical fruits, the antioxidant activity of Karanda fruits was relatively high. The ripe fruits create tarts, curries, puddings, and chutney (5,6). When fruit is slightly under-ripe, it transforms into jelly. In India, pickles are made with green and sour fruits. After removing the peel and seeds and seasoning the fruit with sugar and cloves, they replaced apples with tarts (7)(8). The unripe fruit is utilized as a medicinal astringent. The ripe fruit is consumed as an antiscorbutic and nausea cure (9). The fruits have been used as tanning and dyeing agents. It has been stated that an ethanolic extract from the plant's root can be used to assess the severity of snake venom poisoning (10). The primary objective of this study is to assess the preliminary phytochemical screening of the methanol extract of *Carissa carandas* leaf and to identify the bioactive components of *C. carandas* methanol leaf extract using TLC and GC-MS analysis. There is increasing evidence connecting a medicinal plant's bioactive components to its pharmacological effects.



Figure 1: *Carissa carandas* leaf

2. METHODS AND MATERIALS

2.1. Collection and authentication

Carissa carandas leaves were collected in and around Thirukkalukundram, Chengalpattu district, Tamilnadu, India, from April to June. The specimen of *Carissa carandas* leaves was authenticated with register no 343.19082201 by Dr. Sunil Kumar, a taxonomist at the Siddha Central Research Institute in Arumbakkam, India. The chemicals used for this study were acquired from SISCO Research Laboratories Pvt. Ltd. (SRL).

2.2. Preparation of the Extract

The leaf of *Carissa carandas* was gathered, weighed, washed, and air-dried for 15 to 20 days at room temperature. Pet ether was used to remove fat from the powdered sample. The residue was then extracted with methanol by using the Soxhlet apparatus (11)(12). The methanol extract was concentrated using a rotary evaporator after being filtered through folded paper. The extracts were then collected and stored in glass bottles at room temperature. This methanol extract was undergone phytochemical examination, TLC analysis, and GC-MS analysis.

2.3. Preliminary phytochemical screening

The qualitative analysis was conducted to determine the chemical constituents present in the plant material, including alkaloids, glycosides, tannins, flavonoids, steroids, and terpenoids (13–15)(16)(17). All tests with *Carissa carandas* methanol extract were conducted to various qualitative analyses to discover phytoconstituents.

2.4. Thin layer chromatography

Preparation of TLC plate:

The glass slides were cleaned and dried in a hot air oven. The plates were produced with Silica gel 'G' by weighing 30 g of silica gel and dispersing it in 60 ml of distilled water to form a homogeneous solution. A single drop of the slurry was deposited on the glass slide and then splattered all over to create a thin film (18). Plates were dried in a hot air oven for 30 minutes at 110°C.

Sample application:

1-10 μ l of solvent was typically applied to the origin of a TLC plate using a capillary tube, which was then marked 2 cm from its base (19).

Development of chromatogram:

Samples were applied to plates, which were then put in a TLC glass chamber until the solvent was completely saturated. The mobile phase was then allowed to penetrate the adsorbent phase to within three-quarters of the plate's depth. The dish was removed from the tank and dried in the open air. A U.V. lamp examined the plate at the wavelength of 365nm.

Spot visualization:

The plates were sprayed with a reagent carefully. The plate was then examined using an ultraviolet lamp with a wavelength of 365nm. The colors of fluorescence became visible and were recorded.

GCMS- analysis

In the analysis, a Clarus 680 Gas chromatography equipped with a fused silica column containing Elite-5Mass spectroscopy and Helium as the carrier gas at a constant flow rate of 1 ml/min was used to separate the constituents (20,21). During the chromatography run, the temperature of the injector was set to 260°Celsius. 1 μ L of extract sample was put into the device, and the following oven temperature was set: 60 °C for two minutes; 300 °C at a rate of 10 °C per minute; and 300 °C for six minutes. These were the mass detector conditions: The temperature of the transfer line was 240 °C, the temperature of the ion source was 240 °C, the ionization mode electron impact was 70 eV, the scan period was 0.2 seconds, and the scan interval was 0.1 seconds for fragments between 40 and 600 Dalton (22). Comparing the component spectra to a database of known component spectra from the GCMS NIST (2008) library.

Identification of components

To interpret a mass spectrum from GC-MS, we used the National Institute of Standard and Technology database (NIST), which has more than 62,000 patterns. The spectrums of the known components stored in the NIST collection were compared to the spectrums of the unknown components. The components of the test materials' names, molecular weights, and structures were identified.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Carissa carandas methanolic extracts were tested for the qualitative assessment of alkaloids, flavonoids, cardiac glycosides, tannins, terpenoids, anthraquinone, saponins, reducing sugars, and amino acids using the protocols described elsewhere. Results are shown in Table 1. Some of the phytochemicals were present in the methanolic extract of *Carissa carandas* leaf.

Table 1: Qualitative analysis of a methanolic extract of *C. carandas* leaf

S. No	Secondary Metabolite	Methanolic extract
1.	Alkaloid	+
2.	Flavonoid	+
3.	Glycosides	+
4.	Tannins	+
5.	Saponin	+
6.	Terpenoids	+
7.	Carbohydrate	+
8.	Amino acid	+

Thin layer chromatography

The TLC analysis revealed that methanol was employed for extraction; the high polarity solvent methanol extracted a greater number of secondary metabolites of medicinal value, such alkaloids, flavonoids, glycosides, saponin, and terpenoids, from the leaves of *Carissa carandas*. The chromatographic procedure is the most widely utilized technique for separating plant elements, out of the various possible techniques. For the methanol extract, suitable mobile phases with the right quantities have been determined. The Rf values related to numerous secondary metabolites are listed in Table 2.

Table 2: TLC analysis of *C. carandas* leaf

S. No	Phytochemical	Mobile phase	Spraying agent	Rf value
1.	Alkaloids	CHCl ₃ : Methanol (3:2)	Dragendroff's reagent	0.8
2.	Flavonoids	EA: Methanol : H ₂ O: Glacial acetic acid	10% H ₂ SO ₄ /5% FeCl ₃	0.86
3.	Glycosides	Ethyl acetate: Methanol (1.3:0.5)	Kedde reagent	0.7
4.	Saponin	CHCl ₃ : Methanol (1.2 : 0.2)	Vanillin H ₂ SO ₄	0.71
5.	Terpenoids	Pet ether: Ethyl acetate (2: 0.5)	Vanillin phosphoric acid reagent	0.91

GCMS - analysis

The G.C. fractions of the methanolic extract of *C. carandas* were used to identify various chemicals based on the GC-MS results. These chemicals were identified using M.S. in conjunction with G.C. (Table. 3) summarizes the present study's findings. Dr. Duke's Phytochemical and Ethnobotanical Databases inform the prediction of the molecule. GC-MS analysis of the *Carissa carandas* leaf methanol extract fractions revealed six phytoconstituents. In methanol fraction, the compounds such as a) Methylene chloride b) 2-vinyl-9- β -D-Ribofuranosyl c) 3-O-Methyl-D-glucose d) Hexanoic acid, 5-Methyl-3-oxo-,methyl ester e) 3-Methylmannoside f) Octadecanoic acid, 2-propenyl ester showed prominent peak in the analysis. Six compounds were present, as shown by the GC-MS spectrum profile (Fig. 2), with retention times of 3.013, 20.831, 21.396, 21.986, 22.791, and 31.980, respectively. (Fig. 3 a–f) shows the component fragmentation on an individual basis.

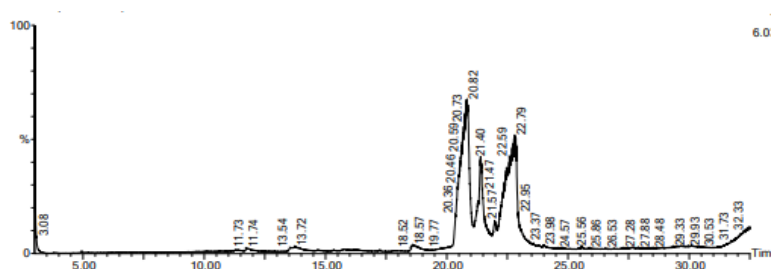
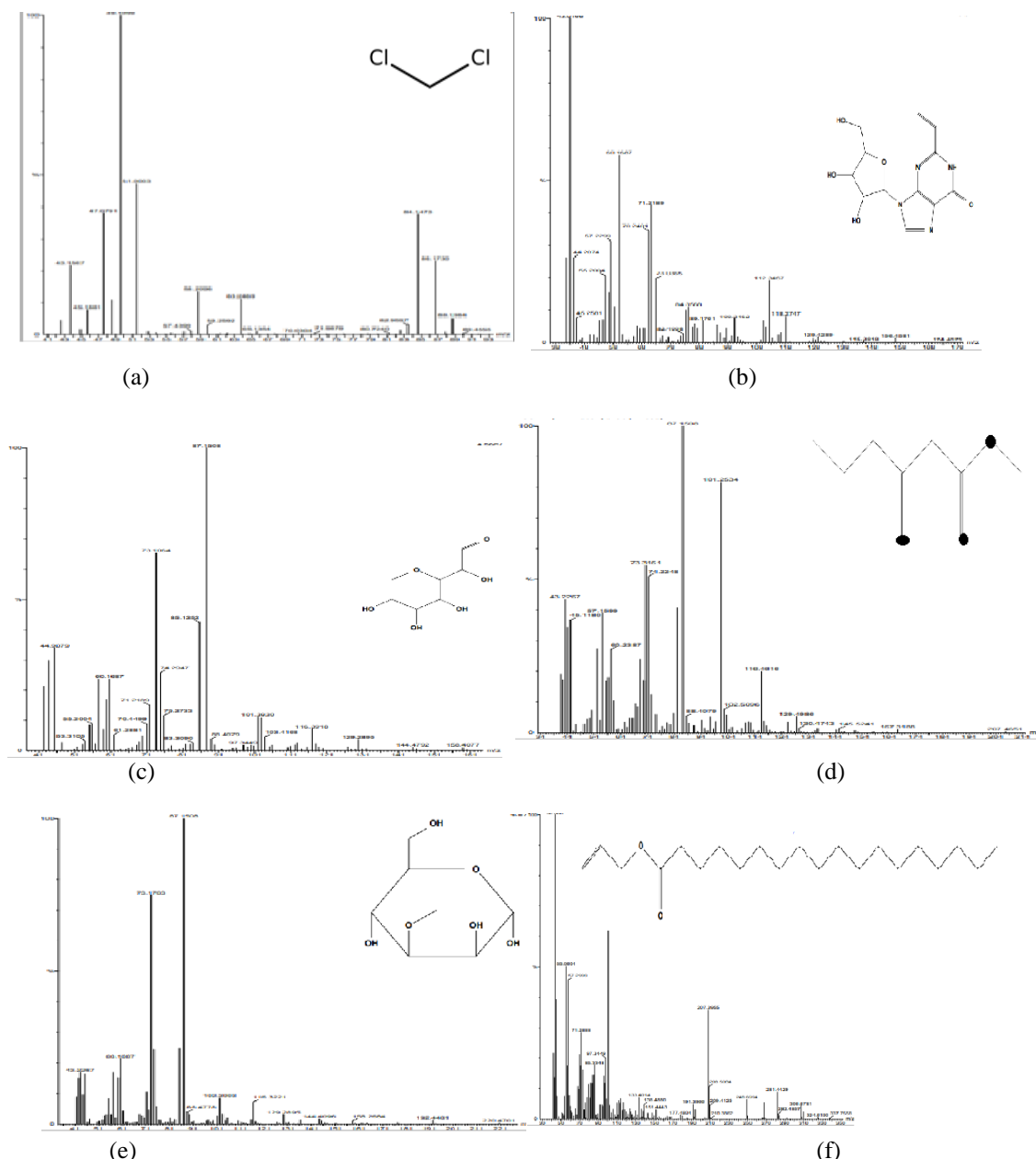


Figure 2: Chromatogram of a methanolic fraction of *C. carandas* leaf extract



carandas plant leaf. This is according to the results of the screening of secondary metabolites in the plant's leaves. There are various strategies for separating plant components; however, the chromatographic approach is the most widely used for general use. The methanolic leaf extract of the studied species, *C. carandas*, contained active metabolites, according to recent TLC experiments. The TLC analysis of the methanolic leaf extract of *C. carandas* revealed the presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, and a variety of other compounds in various solvent compositions, and its Rf values are noted. The Rf values are alkaloids (0.8), flavonoids (0.86), glycosides (0.7), saponins (0.71), and terpenoids (0.91) respectively.

In GC-MS investigation six different components were identified in the methanolic extract of *Carissa carandas* leaf. The retention time and abundance vary between each peak. Retention time revealed the nature of the chemical (23). Higher polarity compounds could be discovered sooner than less polar compounds because they interact differently with the stationary phase of the gas chromatography column. Furthermore, the compound with the lower boiling point was found on the GC MS sooner than the compound with the higher boiling point. Leaf extract has a 3-30 minute retention period. The first peak, which was read as methylene chloride, was found at minute 3.013; the last peak, which was read as 2-propenyl ester octadecanoic acid, was found at minute 31.980. Six substances were found in the *C. carandas* methanolic extract. Six major compounds were identified by the GC spectral analysis, including a) Methylene chloride b) 2-vinyl-9-[.Beta.-D-Ribofuranosyl] 3 O-Methyl-D-Glucose (c) d) Octadecanoic acid, 2-propenyl ester e) 3-Methylmannoside f) Hexonoic acid, 5-Methyl-3-oxo-,methyl ester. Two of the detected phytoconstituents, 2-propenyl ester octadecanoic acid and 3-O-Methyl-D-glucose, are said to have anticancer properties.

CONCLUSION

The description above makes clear that a number of the compounds present in *C. carandas* leaf extracts have therapeutic applications and can be used ethnobotanically to treat a range of illnesses. By using GC-MS analysis, it was possible to determine the relevance of many substances with various chemical structures. The use of *C. carandas* for a variety of diseases by traditional practitioners is confirmed by the presence of many bioactive chemicals, and their diversity and detailed phytochemistry may bring new knowledge to the knowledge in traditional medicinal systems. However, isolating particular phytochemical elements might result in the discovery of a novel drug.

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CONFLICT OF INTEREST

There is no conflict of interest

ETHICAL APPROVAL

This study does not involve experiments on animals and human subjects

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