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Original Article

Phytochemical and biological evaluation of *Carissa macrocarpa*: GC-MS, phenolic analysis, amino acid profiling, and cell studies

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ABSTRACT

Carissa macrocarpa (Natal plum) is a medicinal plant known for its rich phytochemical composition and therapeutic potential. This study evaluated the phytochemical profile and biological activity of its leaf extracts using GC–MS, HPLC, amino acid analysis, and in vitro cytotoxicity assays. GC–MS analysis of the n-hexane extract revealed several bioactive constituents, predominantly fatty acids and their derivatives, including hexadecanoic, octadecenoic, and linoleic acid esters. One major compound accounted for 92.5% of the total extract composition. HPLC analysis identified key phenolic compounds, including gallic acid, caffeic acid, and quercetin, indicating significant antioxidant potential. Amino acid profiling detected seventeen amino acids, including essential amino acids such as leucine, valine, and phenylalanine, highlighting the plant's nutritional significance. The cytotoxic activity of the extract was evaluated against A549 human lung carcinoma cells using the MTT assay. Treatment resulted in a dose-dependent decrease in cell viability, with significant cytotoxic effects observed at higher concentrations. Morphological changes, including membrane blebbing and cell shrinkage, suggested apoptosis as a possible mechanism of cell death. Statistical analysis demonstrated significant differences between the treated and control groups ($p < 0.05$). Overall, *C. macrocarpa* exhibited a diverse phytochemical composition and notable cytotoxic activity, likely attributable to the combined effects of its bioactive constituents. These findings support its potential as a promising natural source of anticancer compounds. Further studies are required to isolate the active constituents and elucidate the molecular mechanisms underlying the observed biological effects.

Keywords: *Carissa macrocarpa*, Phytochemicals, HPLC analysis, Amino acid profiling, Anticancer activity, Apoptosis

Introduction

Carissa macrocarpa (Natal plum), a member of the Apocynaceae family, is a medicinal plant native to South Africa and widely cultivated in tropical and subtropical regions. It is an evergreen spiny shrub with glossy leaves, fragrant white flowers, and edible red fruits used in food products and traditional medicine. The species is highly adaptable to adverse environmental conditions, including saline soils and coastal climates [1-3].

Medicinal plants continue to play a vital role in healthcare, particularly in developing countries, and have attracted growing scientific interest as sources of bioactive compounds with potential therapeutic applications [4-10]. C. macrocarpa is rich in secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds [11, 12]. These phytochemicals are associated with various biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer effects, making the plant a promising candidate for further pharmacological investigation [4, 5, 11-15].

Phenolic compounds are important plant metabolites known for their strong antioxidant activity, protecting cells against oxidative stress and related diseases. They also contribute to plant defense mechanisms and have been associated with a reduced risk of chronic conditions such as cardiovascular

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diseases, diabetes, and cancer [16, 17]. Due to their biological significance, accurate identification and quantification of phenolics are essential. High-performance liquid chromatography (HPLC) is widely used for this purpose because of its high sensitivity, precision, and reliability in analyzing complex phenolic profiles [18, 19].

In vitro cell line models are essential tools in modern pharmacological studies, providing controlled conditions for evaluating the cytotoxic and anticancer activities of plant-derived compounds [20-24]. Previous research has shown that *Carissa macrocarpa* extracts possess notable cytotoxic and antioxidant properties, supporting their potential therapeutic value [25-32]. These findings highlight the importance of integrating chemical profiling with biological assays for comprehensive evaluation of medicinal plants.

Despite the recognized importance of *Carissa macrocarpa*, there is still a lack of integrated studies that simultaneously evaluate its phytochemical composition and biological activity using advanced analytical techniques. In particular, limited research has focused on the combined application of GC-MS for compound identification, HPLC for phenolic and amino acid profiling, and in vitro cell line studies for biological evaluation [33-43]. Therefore, a comprehensive approach is necessary to fully understand this plant's potential.

The present study aims to bridge this gap by conducting a detailed phytochemical and biological investigation of *Carissa macrocarpa*. This includes GC-MS analysis for compound identification, HPLC-based determination of phenolic compounds, and evaluation of biological activity using cell line models. Such an integrated approach is expected to provide valuable insights into the chemical composition and therapeutic potential of this plant, supporting its future applications in pharmaceutical and nutraceutical fields [44].

Materials and Methods

Instruments and equipment

The instruments used in this study included a rotary evaporator (BÜCHI Rotavapor R-205), an electrical balance (ADAM AFP-360L), a magnetic stirrer (Heidolph, Germany), an oven and a water bath (Memmert, Germany), and a UV spectrophotometer (Shimadzu, Japan). For cell culture work, additional equipment included a CO_2 incubator, a laminar flow hood, an inverted microscope (Nikon, Japan), micropipettes, and a microplate reader.

Chemicals and reagents

All reagents employed in this study were of analytical grade. Extractions and analyses utilized methanol, ethanol, chloroform, hexane, petroleum ether, and HPLC-grade acetonitrile. Furthermore, cell culture procedures involved Minimum Essential Medium (MEM), fetal bovine serum (FBS), penicillin, streptomycin, the MTT reagent, and dimethyl sulfoxide (DMSO) as essential experimental components.

Plant material

In November 2024, Carissa macrocarpa leaves were harvested in Tikrit, Iraq. The samples underwent cleaning, shade-drying, and mechanical milling for pulverization. Subsequently, the resulting fine powder was stored in hermetically sealed containers to maintain stability until further experimental analysis.

Extraction method

The extraction process used cold maceration, in which 100 g of pulverized Carissa macrocarpa foliage was immersed in 1500 mL of 90% methanol. This suspension was maintained at ambient temperature for 72 hours, with periodic agitation. Subsequently, the mixture was filtered, and the resulting filtrate was concentrated by rotary evaporation under reduced pressure, yielding the desired crude methanolic extract for further experimental analysis and evaluation.

For GC–MS analysis, a separate portion of the powdered plant material (100 g) was extracted using n-hexane following the same maceration procedure. Following filtration, the hexane extract was vacuum-concentrated to yield the crude material required for subsequent experimental evaluation and analytical investigation in this study.

Amino acid analysis

The amino acid composition was determined via an automated amino acid analyzer. The extract was derivatized using OPA and injected into a C18 column.

Seventeen amino acids were identified (from the chromatogram), including:

Aspartic acid, glycine, lysine, serine, threonine, isoleucine, alanine, valine, tyrosine, arginine, cysteine, methionine, proline, histidine, leucine, glutamic acid, and phenylalanine.

Cell Line and culture conditions

Cell lines used**

A549: Derived from the pulmonary tissue of a 58-year-old Caucasian male diagnosed with carcinoma, this epithelial cell line serves as a critical biological reference. It facilitates rigorous testing and calibration within ISO 17025-certified facilities, enabling researchers to evaluate assay sensitivity, linearity, and specificity while effectively validating or comparing analytical methodologies during implementation and routine performance assessment [45].

Assay Used**: [MTT assay]

Table 1 shows the instruments used in this study.

Table 1. Instruments

No.	Item	Company	Country
1	Incubator	Cypress Diagnostics	Belgium
2	Microtiter reader	Gennex Lab	USA
3	Laminar flow hood	K & K Scientific Supplier	Korea
4	Micropipette	Cypress Diagnostics	Belgium

5	Cell culture plates	Santa Cruz Biotechnology	USA
6	Inverted microscope	Nikon	Japan

Procedure

Cell culture conditions

Cell lines were maintained in MEM (US Biological, USA) supplemented with 10% (v/v) fetal bovine serum (Capricorn-Scientific, Germany), 100 IU/mL penicillin, and 100 µg/mL streptomycin (Capricorn-Scientific, Germany). Cultures were incubated at 37 °C in a humidified environment, and cells in the exponential growth phase were selected for all experimental procedures [46].

MTT procedure

One hundred thousand cells per milliliter were plated (10,000 cells per well) in a 96-well microtiter plate (NEST Biotech, China) and cultured at 37 °C for 72 h until a confluent monolayer formed. Cytotoxicity was evaluated using the MTT assay (Elabscience, China). Cells were treated with serial concentrations of the test compound (1000, 500, 250, 125, 62.5, and 31.2 µg mL^{-1}). Following a 72-h exposure, 28 µL of a 2 mg mL^{-1} MTT solution was added to each well, and the plates were incubated for an additional 3 hours. Subsequently, 100 µL of DMSO was introduced and allowed to solubilize the formazan crystals for 15 min. Absorbance was recorded at 492 nm with a plate reader, and percent cytotoxicity was computed using the standard formula:

The cytotoxicity percentage was calculated using the formula [(OD control – OD sample) / OD control] × 100, where OD represents the mean optical density of the respective experimental wells [47].

Statistical analysis

Each experiment was conducted in triplicate, and the findings were presented as mean ± standard deviation. Using GraphPad Prism, Tukey's post-hoc test evaluated statistical differences, where outcomes reaching a p-value below 0.05 were established as being statistically significant throughout.

GC-MS analysis

The volatile and semi-volatile constituents of the methanolic extract were characterized using gas chromatography-mass spectrometry (GC-MS).

According to the chromatogram and peak report:

- Analysis was performed using a GC-MS system (Shimadzu GCMS-QP2010 Plus).
- The column oven temperature was initially set at 80°C (1 min) and increased to 280°C at 10°C/min, with a final hold of 7 minutes.
- The injection temperature was 280°C, and the ion source temperature was 200°C.
- Helium was used as the carrier gas at approximately 1.46 mL/min.
- Mass spectra were recorded in scan mode over the m/z range 40–800.

The chromatogram (page 1) shows multiple peaks, with a major peak at ~19.63 min, representing ~92.5% of the total area, indicating a dominant compound in the extract.

Identified compounds included:

- Fatty acids (e.g., hexadecanoic acid (palmitic acid))
- Unsaturated fatty acids (e.g., octadecenoic acid derivatives)
- Esters (e.g., linoleic acid esters)
- Alcohols and hydrocarbons

These derivatives exhibit significant pharmacological potential, including antioxidant and antineoplastic properties.

Results and Discussion

Amino acid detection

A novel analytical approach was established to quantify amino acid concentrations within Carissa macrocarpa foliage. Given the prevalence of signal overlapping in chromatographic datasets, this methodology successfully resolves complex chromatograms while accurately determining individual amino acid levels. Validation through quintuplicate analysis of three spiked samples demonstrated robust recovery rates. Typically, evaluating amino acid profiles requires protein hydrolysis, followed by precise chromatographic separation and detection. Currently, no single-step hydrolysis protocol facilitates the quantitative liberation of all proteinogenic amino acids; consequently, three distinct hydrolysis procedures are necessary for comprehensive quantification. Although each hydrolysis phase requires 24 hours, the process permits parallel treatment of hundreds of samples, shifting the primary bottleneck for high-throughput analysis to the chromatographic run time. The proposed method demonstrates speed, sensitivity, precision, and accuracy comparable to those of techniques that utilize sophisticated mass spectrometry. Furthermore, this approach offers significant economic advantages, including lower instrumentation and operational expenses, making it a highly viable tool for the high-throughput characterization of amino acid profiles in diverse botanical, feed, and food matrices. By optimizing the analytical workflow with this refined technique, laboratories can achieve high-resolution data without the prohibitive costs of high-end equipment, ultimately facilitating broader routine screenings and more extensive nutritional assessments across various agricultural and scientific research applications **(Table 2)**.

Table 2. Retention time of amino acid detection in *Carissa macrocarpa* leaves.

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Amount (g/100 gm)	Calculation	Peak type	Compound Name

1	2.01	4012.6	605.4	16.35	Calibration carve	Order	Aspartic acid
2	2.66	3980.1	431.6	25.00	Calibration carve	Order	Glycine
3	3.17	6012.6	794.1	22.45	Calibration carve	Order	Lysine
4	3.92	5032.4	786.5	26.90	Calibration carve	Order	Serine
5	4.12	5965.0	831.9	21.45	Calibration carve	Order	Threonine
6	5.28	4152.6	596.7	22.90	Calibration carve	Order	Isoleucine
7	6.14	5478.9	784.8	27.44	Calibration carve	Order	Alanine
8	7.02	4130.0	387.4	33.98	Calibration carve	Order	Valine
9	7.63	3952.6	549.6	21.41	Calibration carve	Order	Tyrosine
10	8.28	6521.4	828.1	25.70	Calibration carve	Order	Arginine
11	8.72	9014.8	815.6	22.06	Calibration carve	Order	Cysteine
12	9.52	4562.9	284.3	18.70	Calibration carve	Order	Methionine
13	10.08	9214.9	747.2	20.32	Calibration carve	Order	Proline
14	11.72	5362.9	412.6	27.09	Calibration carve	Order	Histidine
15	12.08	6021.4	714.9	29.00	Calibration carve	Order	Lucien
16	12.71	10223.6	715.4	25.90	Calibration carve	Order	Glutamic acid
17	13.93	2521.4	365.8	36.09	Calibration carve	Order	Phenylalanine

An analysis of *Carissa macrocarpa* leaf extract using an amino acid analyzer identified seventeen distinct amino acids, comprising both essential and non-essential varieties. Notably, phenylalanine, leucine, valine, glutamic acid, and arginine appeared in the highest concentrations. These findings highlight the significant biochemical potential and nutritional profile inherent in this plant species, underscoring its overall biological value.

The identification of vital amino acids, including leucine, valine, isoleucine, and phenylalanine, underscores Carissa macrocarpa as a valuable dietary protein supplement. These compounds are fundamental to protein biosynthesis, physiological repair, and metabolic homeostasis. Specifically, the branched-chain amino acids, leucine and valine, exert a significant influence over cellular energy pathways and metabolic regulation. Consequently, these nutritional components are likely to underpin the distinct biological activities and therapeutic potential identified throughout this research study [48-52].

Overall, the amino acid profile of *Carissa macrocarpa* supports its dual role as both a nutritionally valuable plant and a potential contributor to its observed pharmacological effects. The combination of essential amino acids and bioactive metabolites suggests that the plant extract may exert its biological activity through multiple biochemical pathways. Further studies are required to precisely quantify individual amino acids and investigate their specific roles in the observed cytotoxic mechanisms.

Cytotoxic assy

- Observations**

1. ***Cell Morphology analysis:* Cellular manifestations like shrinkage, blebbing, and apoptotic body formation are salient indicators of programmed cell death.
2. ***Cell density analysis:* Reduced cell density relative to control cultures may indicate underlying cytotoxic activity.

- Data analysis

- *IC50 value:* The IC50 metric denotes the specific inhibitory concentration required to diminish a biological or biochemical process by precisely half. In cytotoxicity evaluations, this value represents the concentration of a pharmacological agent required to reduce cell viability by 50% relative to the control group. Consequently, it serves as a fundamental standard for assessing compound potency.
- *Percentage cell viability:* The ratio of healthy cells in a sample to those in a control sample, usually expressed as a percentage. In a cytotoxicity assay, it's used to determine the cytotoxic effect of a test compound. A decrease in cell viability indicates that the compound is cytotoxic.

1. **Inhibitory curve**

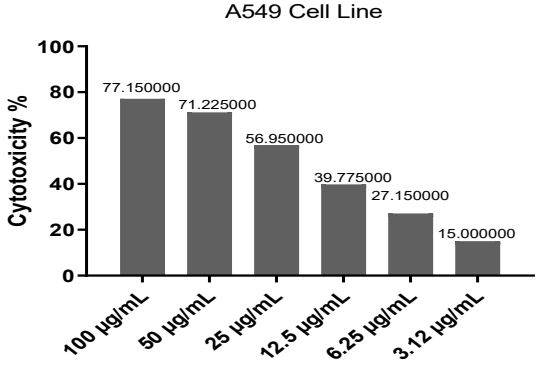


Figure 1. **Inhibitory Curve**

Figure 1 illustrates the inhibitory response of the plant extract regarding cell viability. The horizontal axis displays the test compound concentration (µg), whereas the vertical axis quantifies cytotoxicity as a percentage of total cell death.

2. [IC50]

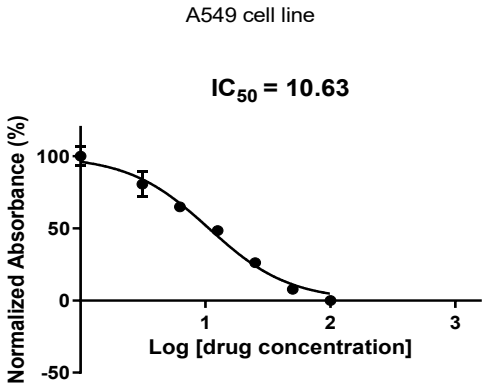


Figure 2. IC50 Curve

Figure 2 shows the dose-response curve, which was utilized to quantify the IC50 value of the plant extract against cancer cells. The x-axis plots the test compound concentration in micrograms, while the y-axis illustrates the percentage of inhibited cells. The IC50 is the logarithm of the concentration of compound X required to reduce overall cancer cell viability by exactly 50%.

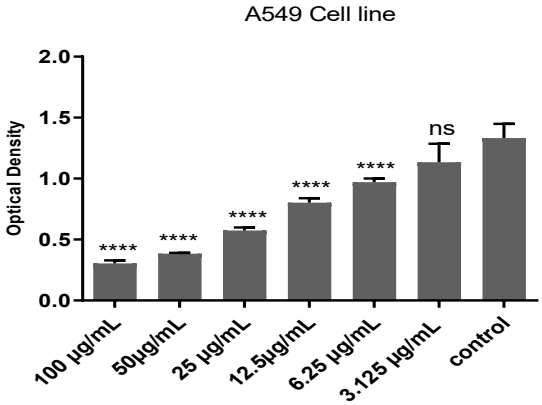


Figure 3. Optical Density Reading Curve

Table 3 shows the concentration with p p-value.

Table 3. Concentration with p-value

Tuke's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
100 µg/mL vs. 50µg/mL	-0.07900	-0.2903 to 0.1323	No	Ns	0.8516
100 µg/mL vs. 25 µg/mL	-0.2693	-0.4807 to -0.05801	Yes	**	0.0091
100 µg/mL vs. 12.5µg/mL	-0.4983	-0.7097 to -0.2870	Yes	****	<0.0001
100 µg/mL vs. 6.25 µg/mL	-0.6667	-0.8780 to -0.4553	Yes	****	<0.0001
100 µg/mL vs. 3.125 µg/mL	-0.8287	-1.040 to -0.6173	Yes	****	<0.0001
100 µg/mL vs. control	-1.029	-1.240 to -0.8173	Yes	****	<0.0001
50µg/mL vs. 25 µg/mL	-0.1903	-0.4017 to 0.02099	No	Ns	0.0905
50µg/mL vs. 12.5µg/mL	-0.4193	-0.6307 to -0.2080	Yes	***	0.0001
50µg/mL vs. 6.25 µg/mL	-0.5877	-0.7990 to -0.3763	Yes	****	<0.0001
50µg/mL vs. 3.125 µg/mL	-0.7497	-0.9610 to -0.5383	Yes	****	<0.0001
50µg/mL vs. control	-0.9497	-1.161 to -0.7383	Yes	****	<0.0001
25 µg/mL vs. 12.5µg/mL	-0.2290	-0.4403 to -0.01768	Yes	*	0.0299
25 µg/mL vs. 6.25 µg/mL	-0.3973	-0.6087 to -0.1860	Yes	***	0.0003
25 µg/mL vs. 3.125 µg/mL	-0.5593	-0.7707 to -0.3480	Yes	****	<0.0001
25 µg/mL vs. control	-0.7593	-0.9707 to -0.5480	Yes	****	<0.0001
12.5µg/mL vs. 6.25 µg/mL	-0.1683	-0.3797 to 0.04299	No	Ns	0.1636
12.5µg/mL vs. 3.125 µg/mL	-0.3303	-0.5417 to -0.1190	Yes	**	0.0016
12.5µg/mL vs. control	-0.5303	-0.7417 to -0.3190	Yes	****	<0.0001
6.25 µg/mL vs. 3.125 µg/mL	-0.1620	-0.3733 to 0.04932	No	Ns	0.1924
6.25 µg/mL vs. control	-0.3620	-0.5733 to -0.1507	Yes	***	0.0006
3.125 µg/mL vs. control	-0.2000	-0.4113 to 0.01132	No	Ns	0.0691

3. **Optical density reading curve**

Figure 3 illustrates the temporal optical density (OD) measurements for cells exposed to plant extracts. The horizontal axis indicates concentration (µg/mL), while the vertical axis displays OD at 492 nm. All data points are presented as the mean ± standard deviation derived from triplicate experiments.

Hplc result

High-performance liquid chromatography (HPLC) analysis was conducted to investigate the phenolic composition of the methanolic extract of *Carissa macrocarpa* by comparing its chromatographic profile with selected phenolic standards, namely gallic acid, caffeic acid, and kaempferol at a concentration of 5 ppm.

The chromatograms of the standards showed single, sharp, well-resolved peaks, confirming their purity and reliability for identification. Gallic acid eluted at 5.88 min, caffeic acid at 9.22 min, and kaempferol at 6.98 min. These retention times served as reference points for identifying similar compounds within the plant extract.

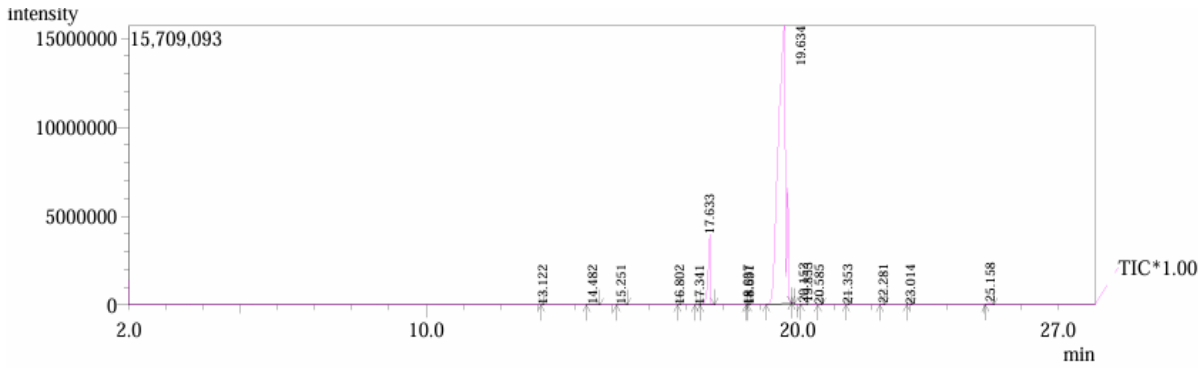
The chromatogram of the methanolic extract of *Carissa macrocarpa* showed a complex pattern with 7 distinct peaks, indicating the presence of multiple phytochemicals. Among these, three peaks were identified by matching their retention times with those of the standards. The peak at 5.82 min closely corresponded to gallic acid (5.88 min), the peak at 6.99 min matched kaempferol (6.98 min), and the peak at 9.25 min aligned with caffeic acid (9.22 min). The minor deviations in retention times (±0.05 min) fall within acceptable experimental limits, confirming the presence of these phenolic compounds in the extract.

Additional unidentified peaks at 4.12 min, 8.02 min, and 11.36 min further highlight the chemical complexity of the methanolic extract. Based on general chromatographic principles, compounds eluting at earlier retention times (3–5 min) are typically more polar, whereas those eluting at longer retention times (8–11 min) are relatively less polar. Accordingly, the unidentified peak at 3.78 min is likely a highly polar compound, while the peak at 11.36 min may correspond to a more hydrophobic constituent.

The identification of gallic acid, caffeic acid, and kaempferol aligns with documented profiles of bioactive constituents in medicinal flora, which are known for their antimicrobial, anti-inflammatory, and antioxidant functions. Consequently, these findings suggest that the methanolic extract of *Carissa macrocarpa* likely exhibits significant biological efficacy, potentially attributed to this specific accumulation of phenolic and flavonoid compounds.

GC-MS analysis of Carissa macrocarpa

GC-MS profiling of the Carissa macrocarpa hexane extract identified a diverse array of bioactive constituents. The chromatogram showed multiple peaks, indicating the presence of various phytochemicals.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	13.122	13.092	13.150	4700	0.00	2121	0.01	2.22	MI	
2	14.482	14.317	14.675	159722	0.07	13114	0.07	12.18	MI	
3	15.251	15.125	15.408	168539	0.07	19236	0.10	8.76	MI	
4	16.802	16.767	16.842	6749	0.00	2377	0.01	2.84	MI	
5	17.341	17.233	17.367	13298	0.01	4624	0.02	2.80	MI	
6	17.633	17.375	17.758	15305285	6.72	3960524	19.82	3.86	MI	
7	18.637	18.608	18.667	5639	0.00	3180	0.02	1.68	MI	
8	18.691	18.667	18.717	3366	0.00	2591	0.01	1.31	MI	
9	19.634	19.150	19.825	210568558	92.50	15617161	78.16	13.48	MI	
10	19.853	19.833	19.917	72185	0.03	29136	0.15	2.52	MI	
11	20.152	20.083	20.258	252592	0.11	68299	0.34	3.72	MI	
12	20.585	20.542	20.658	42037	0.02	15283	0.08	2.80	MI	
13	21.353	21.300	21.383	53438	0.02	25043	0.13	2.13	MI	
14	22.281	22.208	22.342	236724	0.10	69107	0.35	3.42	MI	
15	23.014	22.933	23.075	96665	0.04	22797	0.11	4.22	MI	
16	25.158	25.050	25.275	652464	0.29	126692	0.63	5.15	MI	
				227641961	100.00	19981285	100.00			

Figure 4. GC-MS chromatogram of N-hexane extract

A total of 16 major peaks were detected, with a dominant peak at 19.63 min, representing 92.50% of the total area, suggesting the presence of a highly abundant compound in the extract.

Identified Compounds

The identified compounds mainly belong to:

- *Fatty acids*
  - Hexadecanoic acid (Palmitic acid)
  - Tetradecanoic acid
  - Dodecanoic acid
- *Unsaturated fatty acids*
  - 6-Octadecenoic acid
  - Linoleic acid derivatives
- *Esters*
  - Isopropyl linoleate
  - Oleic acid esters
- *Alcohols and hydrocarbons*

These substances are recognized for their diverse biological properties, which encompass:

- Antioxidant
- Anti-inflammatory
- Anticancer

An investigation using gas chromatography-mass spectrometry (GC-MS) of the Carissa macrocarpa n-hexane extract demonstrated a heterogeneous chemical composition characterized by a wide array of bioactive constituents. The chromatogram **(Figure 4)** showed numerous peaks, indicating a complex mixture of phytochemicals extracted with a nonpolar solvent.

A total of 16 major peaks were detected, confirming the extract's richness in lipophilic constituents. Among these, a dominant peak at 19.63 min accounted for approximately 92.50% of the total peak area. This suggests the presence of a highly abundant compound that may play a significant role in the extract's biological activity.

The primary constituents isolated comprise diverse chemical groups, notably saturated and unsaturated fatty acids, esters, alcohols, and hydrocarbons. Palmitic, tetradecanoic, and lauric acids represent the dominant saturated fatty acids discovered. Frequently observed in botanical extracts, these specific compounds are widely recognized in scientific literature for their significant therapeutic potential, particularly regarding their established antimicrobial and anti-inflammatory biological activities.

In addition, unsaturated fatty acids such as 6-octadecenoic acid and linoleic acid derivatives were detected. These compounds are particularly important due to their well-documented antioxidant and cardioprotective effects. Esters such as isopropyl linoleate and oleic acid esters were also identified, which may contribute to the stability and bioavailability of fatty acids within the extract.

The GC-MS results further confirmed the presence of other compounds, including picolinic acid, succinic acid, and lipid derivatives such as oleic and linoleic acids. These identifications were based on mass spectral matching against standard libraries, providing supporting evidence for the extract's chemical composition.

The predominance of fatty acids and their derivatives in the n-hexane extract is consistent with the nonpolar nature of the solvent, which preferentially extracts lipophilic compounds. This contrasts with polar extracts (e.g., methanolic extracts), which typically contain phenolic compounds such as flavonoids and phenolic acids.

Biologically, the identified compounds exhibit several pharmacological activities. Palmitic acid and lauric acid exhibit antimicrobial and anti-inflammatory effects, while linoleic acid derivatives are known for their antioxidant and anticancer potential. The presence of these compounds suggests that the n-hexane extract of *Carissa macrocarpa* may possess significant therapeutic value.

GC-MS characterization revealed that the n-hexane extract of Carissa macrocarpa contains an abundance of fatty acids and associated derivatives with recognized biological properties. Nevertheless, comprehensive investigations, including isolation, purification, and rigorous in vitro or in vivo testing, remain essential to validate the precise pharmacological activities and potential therapeutic applications of these specific chemical constituents within this plant species.

Conclusion

Overall, *C. macrocarpa* exhibited a diverse phytochemical composition and notable cytotoxic activity, likely attributable to the combined effects of its bioactive constituents. These findings support its potential as a promising natural source of anticancer compounds. Further studies are required to isolate the active constituents and elucidate the molecular mechanisms underlying the observed biological effects.

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Ethics statement: None

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