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**REVIEW OF INTERNATIONAL GEOGRAPHICAL EDUCATION** 

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**Research Article** 

# A Taxonomical Study of Carissa Macrocarpa (Eckl.) A.Dc (Apocynaceae) In Iraq.

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#### Abstract

The current study looks at a number of standardized factors, such as morphological, anatomical, and chemical features, that may be useful in identifying Carissa macrocarpa (Eckl.) A. DC. In terms of microscopical characteristics, Carissa macrocarpa is distinguished by the presence of calcium oxalate crystals in almost all parts of the plant. the phenotypic study included a study of the quantitative and gualitative characteristics of each root, stem, leaf, flower, fruit and seed. It extracted several interesting characteristics that distinguish C. macrocarpa from the rest of the genus Carissa, as well as from the rest of the species belonging to the Apocynaceae, including the presence of hard thorns spread along the length of the plant, which takes the defensive method as well as the Aestivation method by turning the petals lobes towards the left, as well as the length of the petals compared to the petal tube, which is one of the important diagnostic characteristics and was developed as a key to diagnosing the species belonging to the mentioned genus. As for the anatomical aspect, the characteristics of the upper and lower epidermis of the leaf, the floral parts and the epidermis of the stem were measured and described, as well as the transverse sections of each of the leaves and their peduncle and stem, , as well as the study of the venation system in the leaf. It was found that many of these traits are important in diagnosing the species, as it was distinguished by having leaves of the Hypostomatic type, and the transverse section of the Monofacial leaf. The study also dealt with the chemical content of the methanolic extract of the leaf, where the compounds were diagnosed using Gas Chromatography-Mass Spectrometry (GC-MS), It was found that eight chemical compounds are resulting from secondary metabolism, which have an effective role in medical treatments and as a defence mechanism for plants, the most important of which is the glycoside compound.a.-d-6,3-Furanose,methyl-.β.-d-glucohexodialdo-1,4-furanoside, which occupied the highest percentage of approximately 92.83% of the total percentage of the area of Peaks.

#### **Keywords**

Apocynaceae, Carissa macrocarpa, Hypostomatic leaf, thorns, GC-MS.

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## Introduction

Carissa macrocarpa (Eckl.) A. DC. is a species belong to Tribe: Carissege in the family: Apocynaceae, Subfamily: Rauvolfioideae, Order: Gentianales, and Class: Asterids. The name Carissa is probably derived from the Sanskrit Carissa, the name of one of the Indian species. The species macrocarpa is derived from the Greek for large fruit. As for the common names of the species, (Abbas et al., 2014; Sparrow & Hanly, 2002; Wiart, 2006) and Wiart (2006) indicate that it is known as Natal plum in South Africa, in Zulu it is called large num-num, in Uganda it is called Amathungulu, and in Africa, the fruit is called as noem-noem., but the synonym with C.macrocarpa, (Carocho & Ferreira, 2013) mentioned it as (E. Mey) A. DC. C.grandiflora. Carissa is a genus of plants endemic to Africa, Australia, and Asia's tropical and subtropical areas. They are found in two species in South Africa, Carissa macrocarpa and Carissa bispinosa ((Moodley, 2012)), and four species in China (Patel, 2013). Carissa is one of the most beautiful plants offences that can be planted around the fields and gardens in the world, Where (Kumar, Pandey, & Nath, 2007) reported that Carissa seeds have a short shelf life and should be sown immediately after extraction from the (Formiga & dos Santos Isaias, 2011) there are several methods of propagation in plants of the genus Carissa by seed, cuttings, grafting, and pneumatic binding, but these require a season, It is a special agricultural method and takes a long time, as the optimal breeding to produce huge numbers of these plants in a short period is the method of tissue culture. An ethnomedicinal investigation of Carissa species found that distinct morphological parts of the plants are used to treat a wide range of medical problems. The fruit is seen to be high in vitamin C, calcium, magnesium, and potassium(Wehmeyer, 1986). The Zulu people utilize the leaves of C. macrocarpa to cure diarrhoea in cattle, as well as coughs and venereal illnesses (National Research, 2008). In other regions of the world, other Carissa species are also utilized in traditional medicine. Carissa edulis is one of them, and it's utilized in African folk medicine (Ibrahim, Abdurahman, Shok, Ilyas, & Bolaji) (Ibrahim et al., 2007) In Ayurvedic medicine, Carissa carandas is known as (Hegde, Thakker, Joshi, Shastry, & Chandrashekhar, 2009). The fruit is used for many things such as jams and jellies. The Natal Plum is used in guite a few medicinal ways, it is used to prevent anaemia and to build haemoglobin. It is also good for the skin as it prevents pre-mature ageing and offers other benefits as well because fruits contain powerful antioxidants, it protects the heart from disease (Moodley, Chenia, Jonnalagadda, & Koorbanally, 2011). According to a study done in 2011, isolates from the natal plum's stem inhibited proliferation of Leukemia cells, wound healing because of their strong antimicrobial properties. lower your blood pressure (Formiga & dos Santos Isaias, 2011).

# **Materials and Methods**

## **Morphological Study**

Fresh material of Carissa spp. Approximately 30 samples were collected from gardens throughout the college of sciences for women at Babylon University. The Fresh parts of the plant under study were studied using the Olympus Dissecting and Compound Microscope. The exact measurements of the characters were taken using an Ocular Micrometer at a rate of (5-25) measurements for each characteristic. Photographs of some parts were taken on both microscopes using a Huawei plus 10 mobile's camera.

## Anatomical study

Fresh material from leaves, petioles, and stems was fixed for at least 24 hours in formalin acetic acid-alcohol solution (FAA) and stored in 70% alcohol, then sectioned using the handling technique and stained in safranin before being analyzed at the appropriate time. The sections

were examined with a dissecting microscope (Meiji) and photographed with Huawei plus 10 mobile's camera, As previously stated, the stomatal index was computed (Ditcher, 1974). The anatomical terminology used are taken from the following sources. (Ditcher, 1974; Melville, 1976; Esau, 1977)

## Chemical Study

#### **Preparation of the Methanol Extract**

The chemical compounds were extracted from plant leaves according to the method mentioned by (Markham, 1982) with some modifications: The plant leaves were thoroughly cleaned of dust and the parts exposed to bacterial and fungal infections were removed and then left for 10 days at room temperature to dry completely. The leaves were crushed by the electric mill for a period ranging between 5-10 minutes. 2 gm of the ground plant parts were extracted by adding 10 ml of concentrated methanol with continuous shaking for 25 minutes and then left in a dark place at room temperature for a whole day. Filtered by Whatman No.1 type filter paper, To the previous filtrate, a 99% hexane solution was added in a volume of 1 ml to remove the remaining impurities and to concentrate the extract. Suction the separated suspended part by hexane to make it ready for the determination of the active compounds in it.

#### Separation and Identification of Active Compounds

Using a gas chromatography-mass spectrometry (GC-MS) instrument, the active compounds were separated and identified from the crude extract of the plant leaves.

#### **Chemical Compounds Analysis**

The methanol extract of leaves was analyzed by a Japanese-made Shimadzu plus Gas 2010 GC-MS accompanying the Clarus 500 Perkin Elmer system that includes an automatic vehicle identification unit (AOC-20i) and the GC-MS is connected to the MS mass spectrometer according to the following conditions: An Eliter-1 fused silica capillary column (30m X 0.25mm diameter X 1mm thick) made of 100% Dimethylene Polysiloxane that acts as an electron hunting detector. With a constant flow rate of 1 ml/min, helium gas (99.999 percent) was employed as a carrier gas. The device was injected with approximately 2 µl of methanol extract at a split ratio of (1:10). Programmable temperature to 280°C for the injector and 200°C for the ion source. The oven temperature has been programmed at 60°C for two minutes, and an increase of up to 10°C per minute until it reaches 280°C, then 6° per minute until it reaches 300°C for 6 minutes until the end. The mass spectrometry was carried out at a voltage of 70 with a scan-interval of 0.5 s and a splitting rate from 40 to 450 Daltons. The pressure inside the device is 100 kpa at a rate of 1.61 ml/min. The calculated start and end time for the sample device are 32 minutes. Using the TurboMass program version 5.2.0 installed on the device to calculate the mass Spectro product for each compound as a relative amount of its average peak area over the total area and all this information is programmed directly on the device for the plant sample.

## **Result and Discussion**

#### Morphological Study

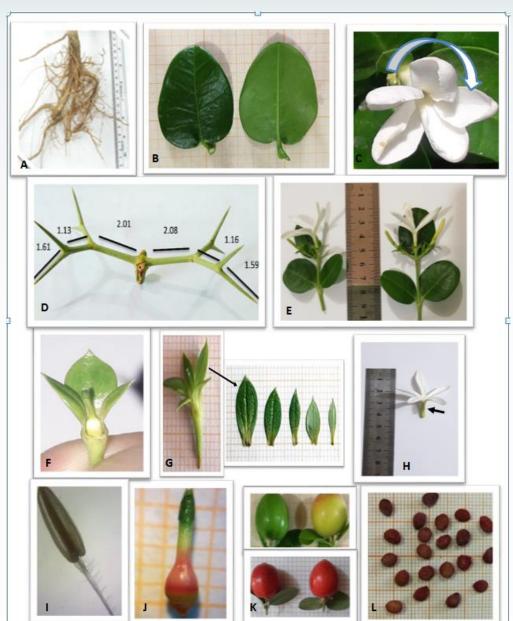
Perennial Shrubs with milky sap, 27-210 cm high, densely branched. Spines are once or twice forked, 6.9-14.5 cm, strong. Leaf-blade simple, opposite, decussate, broadly ovate, Elliptical, Oblong with an entire margin, Adaxial surface has a dark green colour, while abaxial surface is a

lighter green, 2.1-6.6 × 5.8-1cm, thickly leathery, Hairy, symmetric base rounded to obtuse, apex mucronate to Acute, lateral veins obscure and pinnately reticulate venation. cymes terminal and Axillary, (1–)3-flowered. Pedicel 5–6 mm. Flowers fragrant. Sepals very narrowly ovate, 6–10 × 2.3– 5 mm. Corolla white, tube 1.2–1.6 cm, pubescent inside; lobes oblong, 2.2–3 cm, overlapping to left. The androecium consists of free five stamens, anther in length (2.08-2.66) mm, and in width (0.53-0.87) mm, Fertile, the top and base of the anthers appeared in a rounded shape-acute, (3.32-7.78) mm length of filaments, the thread was distinguished by being of the hairy type, the length of the filament ranged between (0.07-0.33) mm. The gynoecium: Bicarpellary, syncarpous, two-locules, Each locule contains one to several ovules, Axial placentation, style narrow, total length (5.6-1.4) mm, stigma branched, (0.3-2.9) mm in length; ovary superior, (1.12-1.46) mm long, (0.44-0.68) mm wide. Fruit is large, bright red when ripe containing latex in the skin and very edible indeed, sweet flavour, Simple Fruits Succulent and Berry, ovoid, 1.8–3.8 cm, ca. 4-23-seeded. The flowering period from March to May, Figure (1). These characteristics were emphasized by (Allam, Abd El-Kader, Mostafa, & Fouad, 2016; Singhurst & Holmes, 2010). This study is consistent with that of (Leeuwenberg & Van Dilst, 2001; Lim, 2012; Malik, 2010; Moodley, Koorbanally, & Jonnalagadda, 2012; Nedi, Mekonnen, & Urga, 2004; Wiart, 2006) as being shrubby perennials while described by (Pereira, Barros, & Ferreira, 2016) in the Chinese Encyclopedia as small trees or shrubs. (El-Taher, El-Gendy, & Lila, 2019)) reported that all plants in the Oleander family are perennial and evergreen except for Pachypodium lamerei Drake plants that are deciduous. As for the leaves, their characteristics are similar in the study of (Al Afas, Marron, & Ceulemans, 2006) when mentioned that the leaf took an oval shape, while the study of (Leeuwenberg & Van Dilst, 2001) showed that the leaf blade has many forms, including oval, orbicular, ellipsoid, and round, while (Khan, 2019) study showed that the shape of the blade was elongated, oval and shield, and most likely took the rounded shape, and an obtuse base, which corresponds to the study of the latter, in addition to the addition of two shapes, namely, Cordate and Cuneate, as the current study did not record such shapes. The plant also contained thorns, which are considered a structural defence mechanism, this was stated by (Grubb, 1992) as reported by (Pereira et al., 2016) and the presence of Leafy stipules at the point of branching of the thorns, and as indicated by (Hany E. Khalil, Mohamed, Morsy, & Kandeel, 2018; Khan, 2019; Kumar et al., 2007; Leeuwenberg & Van Dilst, 2001) that they appear on both sides of each node in the Bract axis, and this was also confirmed by the current study.

## Anatomical study

#### The characteristics of the cross-section of the leaf blade and Leaf surface

the outline of a cross-section of the leaf is convex and Monofacial, The mesophyll contains a welldifferentiated palisade, which is made up of palisade cells with 1-3 cell layers on both surfaces of parenchyma cells containing chlorenchyma, which is important for photosynthesis, and spongy layer areas that are less regular, similar to what (Formiga & dos Santos Isaias, 2011) as the palisade parenchyma consists of 3 layers on the upper side and one layer on the lower side of the leaf tissue, and different in that with (Souilem et al., 2018), when they showed that the palisade cells are located under the surface of the upper epidermis of the leaf, meaning that they have Two faces (Bifacial) that contained 1-2 rows. The spongy tissue is composed of parenchyma cells with thin round walls separated by large intercellular spaces. Most of these cells contain Druses crystals of calcium oxalate. The vascular bundle is collateral that forms an open arch composed of xylem on the adaxial and phloem on the abaxial side of the leaf Although (Formiga & dos Santos Isaias, 2011) indicated that the vascular bundle is of the bicollateral vascular bundle. Wood vessels form in the form of rows distributed between the parenchyma cells. The vascular system is represented as an arc-like strand of vascular collateral bundles accompanied with perimedular phloem patches. Spiral and annular lignified thickenings make up the xylem vessels. The cambium is made up of 1-2 rows of tiny cellulose thin-walled cells oriented radially. The phloem tissue is made up of



sieve components and phloem parenchyma and is very soft and compacted.

**Figure (1):** Morphological characters of C. macrocarpa plant (A-Root, B-Leaf, C-Asetvation, D-Spines, E-inflorescences, F-superior ovary flower, G-Sepals, H-Petals, I-Stamine, J-Pistil, K-Fruits, L-Seeds)

Except for a few of the descriptions of isobilateral leaves such as Wrightia saligna (Ngan, 1965), Aspidosperma quebracho and Nerium oleander (Metcalfe & Chalk, 1979). The results of this study and the information of other studies on Apocynaceae leaf anatomy by various authors show that the leaves are dorsiventral (bifacial). Cuticular ornamentation deposited on the adaxial and abaxial surfaces of epidermal cells' outer walls. The epidermal cells on both sides are polygonal to irregular in form, with anticlinal walls that are straight to somewhat undulate. It is characterized by a uniseriate epidermis whose cells are square, compact, interspersed with stomata, surrounded by a thin layer of cuticle on the outside. On the surface, polygonal cells appear, the walls of the

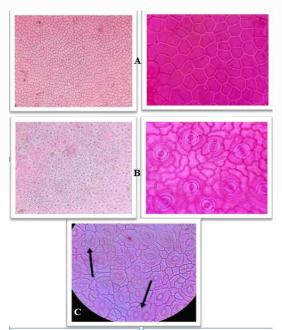
vertical cells are straight, As for the surface covering of cauline leaves, the upper surface and the lower surface are distinguished by the presence of three types of hairs

- 1- Eglandular hairs, with a rounded top and an oval surface
- 2- Multicellular Uniseriatecellular
- 3- The papillae are finger-shaped projections located on the upper surface of the epidermis

These results agree with what was confirmed by previous studies such as Leeuwenberg and (Gabr, Khafagi, Mohamed, & Mohamed, 2015; Khan, 2019; Wink & Van Wyk, 2008). but it does not agree with the study of (Hany Ezzat Khalil, Aljeshi, & Saleh, 2015; Hany E. Khalil et al., 2018). when they indicated the absence of any type of hairs on the surface of the plant. The Leaves were characterized by being of the Hypostomatic type due to the spread of stomata on the Abaxial surface without the upper Adaxial surface, where the contrasting stomata types Anisocytic type and Cyclocytic type appeared. Within the same species, stomatal density can vary between leaves, plants, and individuals (Al Afas et al., 2006) Environmental variables like as light, air humidity, water availability, and atmospheric CO2 concentration can also affect it (Woodward & Kelly, 1995).

**Figure 2:** Cross-sections of a leaf (A - general shape at X4 magnification, B - cross-section of a leaf with X40 magnification, C - vascular bundle in the median vein)

The results of the study also agreed with what mentioned about the presence of stomata in the form of paired pairs surrounded by common auxiliary cells, a characteristic similar to what was reported by (Patel, 2013) when studying the species Carissa carandas. It was found that the largest size of normal upper surface epidermal cells is  $52.5 \times 35 \mu m$  while the smallest size of epidermal cells of the same surface is  $25 \times 17.5 \mu m$ , while the lower surface recorded the largest size is  $70 \times 30 \mu m$  while the smallest size is  $20 \times 10 \mu m$ .



**Figure 3**: Dimensions and shapes of epidermal cells and leaf stomatal complex for the type under study (A- upper epidermis B- lower epidermis C- paired stomatal, 1- magnification under 10X power, 2- magnification under 40X power)

The venation is brochidodromous in this type, since the secondary veins do not end at the edge, but join to produce conspicuous arches, according to (Hickey, 1973) nomenclature for the architecture of dicotyledonous leaves. The lateral veins branch into secondary veins, which in turn into tertiary veins and other smaller veins that do not meet each other, enclosing small areoles between them. This is confirmed by Leeuwenberg and (Wehmeyer, 1986) and both researchers reported that the tertiary veins are less visible than the rest of the veins. As well as what was confirmed by (Khalaf) to agree with the results of the current study.

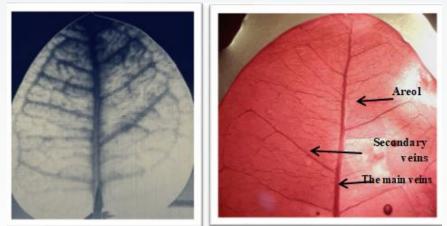
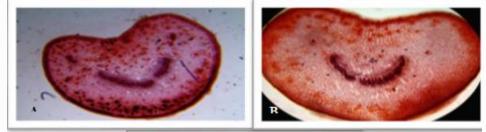


Figure 4: Venation of C. macrocarpa Leaf

## The characteristics of the cross-section of the petiole

The cross-section of the petiole appears the heart of the general shape into a semicircle concave from the upper surface and with prominent grooves on either side of the concave surface with a smooth surface and consists of the same layers as the leaf tissue. The cortical tissue indicates that the structure of the cortical tissue is comparable to that of the leaf in the midrib area. (Fig:5)





**Figure 5:** Transverse sections of a leaf petiole (A - general shape with X4 magnification, B - cross-section with X10 magnification, C - vascular bundle with X40 magnification)

## The characteristics of the transverse section of the stem

The stem had a circular cross-sectional form that was sold, and different sections were produced in different areas of the stem to demonstrate the anatomical properties of the stem and to identify the differences between these parts. Epidermis layer consisting of small cells with a thickness ranging between 12.5-20 µm, characterized by being compact, simple, single-row, rounded to semi-square, as it appears from the outside with a zigzag, with a thick and smooth cuticle. A thin cortex surrounds the epidermis. A continuous ring of vascular tissue is pierced by the medullary rays, encircling a broad parenchymatous pith in the center. The cortex layer comes after the epidermal layer and is composed of several rows of thin-walled lamellar collenchyma cells of a semi-circular shape, characterized by their thick walls, followed by several rows of parenchyma cells, the thickness of their walls being less the further towards the centre of the stem, and the thickness of the cortex varies between 300-400 µm. The cortex follows by a pericycle consisting of rounded parenchyma cells with thick walls. The vascular system consists of a complete ring of collateral vascular bundles with perimedullary phloem. The phloem is made up of sieve tubes, companion cells and phloem parenchyma. It represents the widest area above the xylem than the bottom of the xylem, and the zone of the phloem shows the non-branching laticiferous tubes, responsible for the secretions of white milk that comes out when the stem is broken. Followed by a vascular cambium consisting of 3-4 rows of flexible cells with thin, semi-rectangular, cellulosic, meristematic, tangentially elongated radially arranged cells. The xylem is made up of vessels, wood parenchyma and wood fibres. The xylem vessel has helical and pitted thickenings and is made up of lignified radially organized components. Wood fibres are found in clusters and are fusiform in shape, with a small lumen, sharp apices, and occasional forked apices. Cells with thin, large, polygonal walls to heterogeneous cells make up the majority of the cross-section. The size of the cells grows larger as you go closer to the center. (Fig.6). All of the findings in this study coincided with (Omino, 1996) when stems from 20 Apocynaceae species were studied. The epidermis was on the exterior, followed by the cortex, which occasionally contained single or clustered stone cells; the pericycle, which had white, sometimes mucilaginous, unlignified fibres, often in groups; the phloem; and lastly, the xylem, which forms a continuous cylinder crossed by narrow rays..

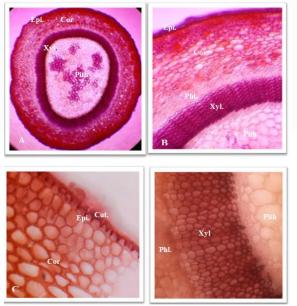


Figure 6: Stem cross-sections (A- transverse section of the stem with X4 magnification, B - stem transverse section with X10 magnification, C - cuticle and epidermis of the stem, D - wooden

vessels in the stem) Epi=Epiderm, Cut=Cuticle, Cor=Cortex, Xyl=Xylem, Phl= Phloem

# Chemical study

GC-MS technique was used to determine the chemical content in the methanolic extract of C. macrocarpa leaves. The results showed that there are clear variations in terms of the number of chemically active compounds registered in the species after confirming them in comparison with the chemical electronic library in terms of retention time, and the area of the percentage curves for each Peak area, Molecular formula, Molecular weight, Chemical composition, and CAS. In general, eight chemical compounds collected in the alcoholic extract of the leaves of the species were observed to have an effective biological importance and according to the retention time per minute by excitation 2.050, 17.384, 17.895, 17.986, 19.287, 21.105, 26.756 and 28,083, respectively with their names Acetaldehyde, methoxy, a-d-6 3,3-Furanose, methyl-β.-dalucohexodialdo-1,4-furanoside, Di(1,2,5-oxadiazolo)[3,4-b;3,4-E]pyrazine,4,8-diacetyl Hydroperoxide, 1,4-dioxan-2-yl, Isoamyl nitrite, 7,8-Dioxabicyclo [4.2.2]dec-9-ene, Cyclopropane carboxylic acid, 4-nitrophenyl ester, 2-(Acetyloxy)-3-[2(6-bromo-2-hydroxy-2,5,5,8atetramethyldecahydro-1-naphthalenyl) ethyl]- (Table 1). The oleander family (Apocynaceae) contained many glycosidic compounds Glucosinolate (Heneidak et al., 2006), and it was found that the alycoside compound in the leaves of the studied species was a.-d-6,3-Furanose, methyl-. β.-d-glucohexodialdo-1,4-furanoside occupied the highest percentage of approximately 92.83% of the total peak area, which indicates the presence of a high percentage of glycosides compared to the compound Di(1,2,5-oxadiazolo) [3, 4-b;3,4-E]pyrazine, 4,8-diacetyl- which represented the lowest percentage of 0.10%. (Figure 7). Several studies such as (Abbas et al., 2014; Patel, 2013; Souilem et al., 2019; Souilem et al., 2018)) confirmed the possibility of employing glycosidic compounds for effective biological activities, including antioxidants, inflammation, cytotoxic activity and bactericides such as E.coli bacteria, and the presence of phenolic compounds was recorded. Various studies by Carocho and (Heade et al., 2009) have a role in the treatment of cancer, added by) to their importance in the treatment of the liver. (Hany E. Khalil et al., 2018). study confirmed, during C.

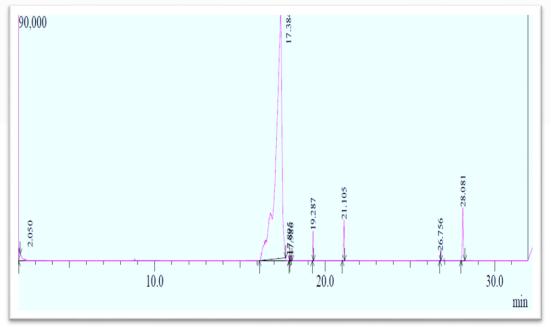


Figure 7: Chromatogram profile for GC-MS of Carissa macrocarpa

macrocarpa leaf extraction and analysis, that three flavonoid glycosides and three phenolic compounds were isolated for the first time from the plant that can molecularly bind with four cancer-inducing enzymes to inhibit the activity of carcinogenic cells. (Martens & Boyd, 1994) explained that the chemical defence method involves the production of chemical compounds either organic such as phenolics and glycosides or inorganic such as heavy metals such as Nickel, It was confirmed by (Pereira et al., 2016) that the plant's defence mechanism is thistles and plant milk, which was referred to in the anatomical study, and (Wink & Van Wyk, 2008) mentioned that plant milk contains cardiac glycosides.

## Conclusions

The current study showed that the perennial nature of the plant enables it to live in different environments within the environmental conditions in Iraq, which made it one of the ornamental plants found in many central islands and home gardens. The study proved that the characteristics are of taxonomic importance in diagnosing the species under study from the rest of the species of the genus Carissa, including the presence of thorns and the way they branch, as well as the method of superimposing petal lobes towards the left as well as the length of the petal lobes, in addition to the quantitative and qualitative variations in the dimensions and shapes of leaves, fruits and seeds. The anatomical variations produced by the study have an important role in enhancing the characteristics of the plant, including the nature of the surface covering and the presence of pink crystals and milk ducts, as well as the nature of the veining of the leaves that distinguish it from the rest of the members of the genus Carissa. The chemical study plays a distinguished role in providing researchers with the nature of chemical metabolites, which contribute to many important biological activities, including the glycoside compounds that appeared in a high percentage within the studied type.

11(7), SPRING, 2021

### Table 1:

Components detected in the methanolic extract of Carissa macrocarpa

No.	Name	Retention Time	Area	Area%	Classification	Mol. Weight g/mol	CAS	Molecular Formula	Table 1: Components detected in the methanolic extract of Structure
1	Acetaldehyde, methoxy-	2.050	13562	0.57	Aldehydes	74.08	10312-83-1	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	0 H 0
2	a-d-6,3-Furanose, methyl-βd- glucohexodialdo-1,4- furanoside	17.384	2190540	92.83	Glycosyl compounds.	192.17	0-00-0	C7H12O6	
3	Di(1,2,5-oxadiazolo) [3,4-b;3,4-E] pyrazine, 4,8-diacetyl-	17.895	2451	0.10	Hetero Aromatic and Acetyl	250.17	186205-18-5	C8H6N6O4	
4	Hydroperoxide, 1,4- dioxan-2-yl	17.986	7352	0.31	Organic compounds known as 1,4- dioxanes	120.104	4722-59-2	C4H8O4	HO
5	Isoamyl nitrite	19.287	20953	0.89	Nitrite ester	117.15	110-46-3	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	0 N

6	7,8-Dioxabicyclo [4.2.2] dec-9-ene	21.105	43606	1.85	Dioxa Akene	140.1797	52148-56-8	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	o
7	Cyclopropane carboxylic acid, 4- nitrophenyl ester	26.756	6041	0.26	Ester	207.1828	0-00-0	C10H9NO4	
8	2-(Acetyloxy)-3-[2-(6- bromo-2-hydroxy- 2,5,5,8a- tetramethyldecahydr o-1-naphthalenyl) ethyl]-	28.083	75280	3.19	Ester	487.5	115334-14-0	C24H39BrO5	

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