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Research

Pharmacognostical, Preliminary Phytochemical Probe and Phenetics of *Carissa carandas* (L.) Leaves

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Abstract

The present study aims to conduct a pharmacognostical evaluation of *Carissa carandas* leaves to establish a standard for its identification and medicinal use. *Carissa carandas*, a member of the Apocynaceae family, is traditionally used to treat ailments such as diarrhea, fever, and skin disorders. The study involved the collection and authentication of fresh leaves, followed by organoleptic, macroscopic, and microscopic evaluations. Powder microscopy and physicochemical parameters, including ash values and extractive contents, were also determined. Fluorescence analysis was performed under visible and UV light. The leaf exhibited key diagnostic features such as anisocytic stomata, reticulate venation, and the presence of starch grains and calcium oxalate crystals. Additionally, phenetic analysis compared *C. carandas* with related species to explore genetic diversity. The phytochemical screening confirmed the presence of alkaloids, flavonoids, and saponins, supporting the plant's medicinal potential. These findings provide a comprehensive reference for the identification and quality control of *Carissa carandas* in herbal medicine.

Keywords: Pharmacognostical studies, physico-chemical, phenetics, *Carissa carandas (L.).*

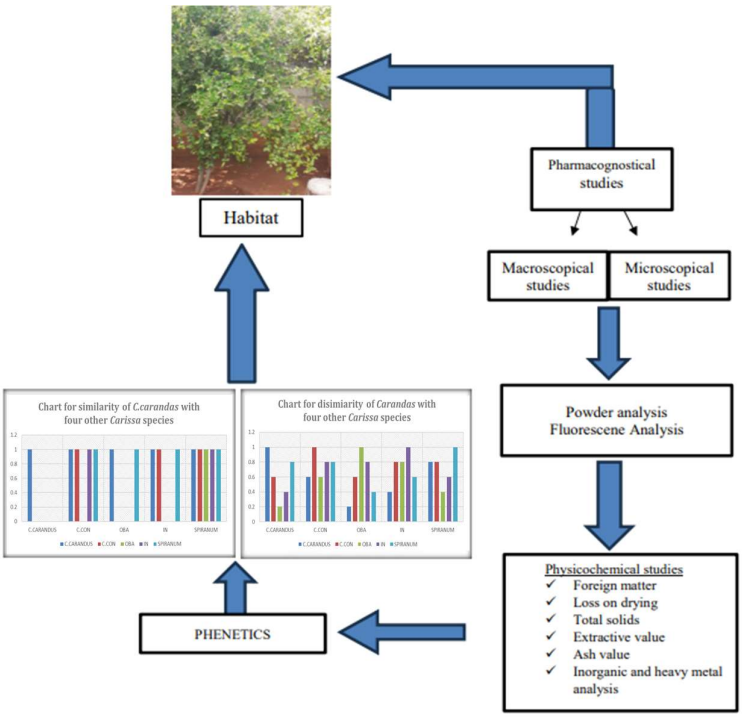
INTRODUCTION

Carissa carandas (L.), commonly referred to as Karonda, Traditionally, various parts of the plant, including the leaves, fruits, roots, and bark, have been utilized in folk medicine to treat a wide range of ailments, including diarrhea, fever, skin infections, respiratory disorders, and gastrointestinal issues. The plant is also consumed as a food source, particularly for its fruits, which are rich in vitamins and nutrients, and are often used in making jams, pickles, and beverages. The plant exhibits significant antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, cardioprotective, anticancer, neuroprotective, analgesic, antipyretic, antispasmodic, anthelmintic, immunomodulatory, and antidiarrheal activities. These pharmacological properties not only support its traditional uses but also open up new avenues for research in drug development.[1-3] In recent

years, *Carissa carandas* has gained scientific attention due to its rich phytochemical profile. Phytochemical screenings have revealed the presence of various bioactive compounds, including alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids.[4] Despite its wide application in traditional medicine, comprehensive pharmacognostical studies that detail its macroscopic, microscopic, and physicochemical characteristics are still lacking. These aspects are crucial for the standardization of herbal medicines, especially for ensuring consistency in commercial formulations. It is immediate and essential to identify the pharmacognostical charecters of the leaves of *Carissa carandas.*[5-7]

Despite its well-documented traditional usage and the existing pharmacognostical studies on *Carissa carandas*, further validation is necessary to confirm and refine these findings, particularly in the context of the geographical characteristics of my region. Establishing the diagnostic features of the plant through detailed macroscopic, microscopic, and physicochemical evaluations, alongside quantitative estimation of phytoconstituents, remains crucial for ensuring the consistency and quality of herbal formulations. This study aims to build upon previous research by validating the pharmacognostical characteristics of *Carissa carandas* leaves, with an emphasis on how these attributes may vary according to the specific geographical conditions of my area, thereby providing additional data to support the standardization of this medicinal plant.

Graphical abstract



MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves of *Carissa carandas* Linn., were collected from domestic garden of Indian Medical Association convention centre, Madurai Medical College, Madurai dist, Tamilnadu during the month of March - 2024 and was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Professor, Department of Botany, American College, Madurai-02. The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical evaluation: Fresh leaves were subjected to pharmacognostical studies. Organoleptic, macroscopy and microscopy of the leaves of *Carissa carandas* were studied.

Organoleptic evaluation: Fresh leaves were collected and checked for their colour, odour and taste by sensory characters.

Macroscopic evaluation: The macroscopic features of the fresh leaves of *Carissa carandas* were studied according to the methods.[8]

Micropscopic evaluation: Fresh hand made sections were taken, stained with routine staining reagent. Transverse

section were photographed using Axiolab 5 trinocular microscope attached with Zeiss Axiocam 208 colourdigital camera under bright field light. [9-10]

Determination of leaf constants

Rectangular cut leaf pieces between margin & mid rib were boiled with saturated chloral hydrate solution until colourless and slides prepared for vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio as per WHO guidelines [11].

Preparation of Powder

The leaves were collected and shade dried, was powdered using blender. The powder was sieved in a No.60 sieve for powder microscopy, physicochemical parameters, and chemical tests. The powder was stored in airtight container and was screened for its microscopic cell characters

Powder microscopy

A trace of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using microscope. [12]

Fluorescence analysis

A small quantity of leaf powder was transferred to test tube and 1-2 drops of freshly prepared various solution was added and colour was observed under visible, UV 254 and UV 365 nm. [13]

Physicochemical evaluation

Total ash, water soluble ash, acid insoluble ash, and extractive value using various solvents, loss on drying, were determined according to the standard procedure [14]. including qualitative determination of heavy metal and inorganic elements was done by the method [15].

Preparation of Hydro-alcoholic Extract of *Carissa carandas (L.)* (HAECC)

The leaves were collected, shade dried and coarsely powdered, passed through sieve no 40, was extracted with ethanol by maceration technique. The content was allowed to macerate in hydro-alcoholic (70:30) for about 72 hours. The extracts were then filtered through Whatmann filter paper NO.42 (125 mm) to remove all non-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness and stored in sterile bottles.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out by using different reagents through standard procedure for identification the presence of phytoconstituents as per standard procedures [16] and is tabulated in Table 6.

Quantitative Estimation of Phyto-constituents

a) Determination of Gallic acid equivalent in HAECC
b) Determination of Tannic acid equivalent in HAECC
c) Determination of Quercetin equivalent in HAECC

Determination of Gallic acid equivalent in HAECC

A series of calibrated 10ml volumetric flask is taken and standard solution (gallic acid) And HAECC solution of various concentrations (5, 10, 15 20µg/ml) is taken. To each of this solution add 5ml of distilled water and 0.5ml of Folin's Ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made up to 10 ml with distilled water. It is allowed to incubate for 2 hours at room temperature. Intense blue colour is developed. The reaction mixture without sample is used as blank. After incubation, absorbance is measured at 725nm using UV spectrophotometer and the mean values will be recorded. The calibration curve will be plotted using standard gallicacid. Total phenolic content of HAECC extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) [17]

Determination of Tannic acid equivalent in HAECC

Prepare various concentration of HAECC into test tubes. To this, 0.5ml of Folin-Denis reagent and 0.8mL of distilled water was added. The tubes were kept aside for 15min. To this, 1mL of sodium carbonate solution was added and the remaining volume was made up with 7.5mL of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannic acid, used as a standard was taken at different concentration 5, 10, 15, 20µg/ml in different test tubes and the procedure adopted above was followed. The

calibration curve for tannic acid was plotted using concentration versus absorbance. A linear regression equation was calculated and the equation was used to calculate the amount of total tannins as tannic acid equivalent. The amount of tannin content is expressed in mg/g of extract. [18]

Determination of Quercetin equivalent in HAECC

Total flavonoid content was measured with the aluminium chloride colorimetric assay. A series of calibrated 10ml volumetric flask were taken and standard solution (Quercetin) and HAECC solution of various concentrations (10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) were taken. To each of these solution add 4ml of water and 0.3ml of 5% sodium nitrite solution is added. After 5 minutes, 0.3ml of 10% aluminium chloride is added. At 6th minute, 2ml of 1M sodium hydroxide is added. Finally, volume is make up to 10ml with distilled water and mix well. Orange yellowish colour is developed. The absorbance is measured at 510 nm spectrophotometer using UV-visible spectrophotometer and the mean values will be noted. The blank is performed using distilled water. The calibration curve is plotted using standard Quercetin. The total flavonoid content in the extract is expressed as milligrams of Quercetin equivalent per gram of extract. [19]

Phenetics

Carissa carandas belongs to the family Apocynaceae. The genus *Carissa* contains 100 to 120 accepted species. Genetic diversity of *Carissa* is high in India.[20]

The following 5 species of *Carissa* were selected for Phenetics.

- *Carissa carandas Linn.*
- *Carissa spiranum Linn.*
- *Carissa congesta Wigh.t*
- *Carissa opaca Stapf ex Haines*
- *Carissa inermis*

The characters such as shape, size, apex, petiole, base of the *Carissa* species leaves are considered.

RESULTS AND DISCUSSION

Macroscopical evaluation

The fresh leaves of *Carissa carandas (L.)* are green in colour, characteristic odour, astringent, simple, oblong and conical, opposite, obtuse or rounded, cuneate or rounded, entire, reticulate venation, measuring 3.4 to 7.5cm length and 2.6 to 4.7 cm width [Table 1 & Fig 1].

Table 1: Organoleptic and Macroscopical studies of *Carissa carandas* (L.)

S.NO	PARAMETERS	OBSERVATION
1	Colour	Dorsal – Dark green ; Ventral – Pale green
2	Odour	Characteristic odour
3	Taste	Astringent
4	Leaf Type	Simple
5	Shape	Oblong and conical
6	Arrangement	Opposite
7	Apex	Obtuse or Rounded
8	Base	Cuneate or rounded
9	Margin	Entire
10	Venation	Reticulate
11	Surface	Dorsal – Leathery ; Ventral - Glabrous
12	Length	3.4 to 7.5cm
13	Width	2.6 to 4.7 cm
14	Petiole length	2 to 5 mm long
15	Stipules	Absent

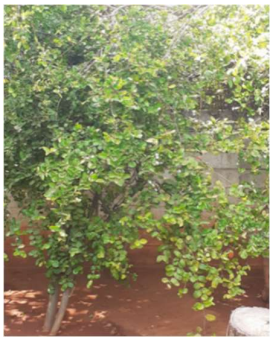


Fig 1.1: Habitat of *Carissa carandas (L.)*

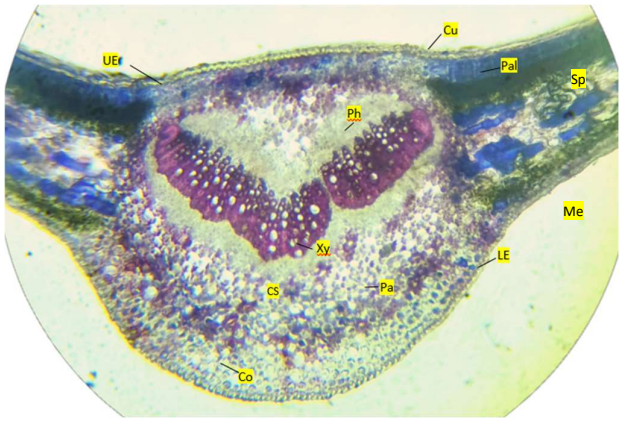
Fig 1.2: Dorsal view of *Carissa carandas (L.)*

Fig 1.3: Ventral view of *Carissa carandas (L.)*

Microscopical evaluation

A transverse section of the leaf of *Carissa carandas* Linn. at the midrib shows a clearly defined single-layered upper epidermis composed of rectangular cells with a thick cuticle. The vascular bundle, encased in a thick layer of collenchyma, is prominently visible. A notable feature is the presence of a single layer of a discontinuous crystal sheath beneath the phloem in the centrally located collateral vascular bundle. In the phloem region, prismatic and rosette crystals are present, along with randomly distributed starch grains.

The leaf lamina comprises a multi-layered mesophyll, including palisade and spongy parenchyma, interspersed with vascular bundles. Both the upper and lower epidermis are covered with a cuticle, with stomata primarily located on the lower epidermis. The palisade parenchyma consists of elongated cells arranged in two or three layers beneath the upper epidermis, in contrast to the loosely arranged spongy parenchyma. Additionally, calcium oxalate crystals and laticifers can be observed in the mesophyll and cortex [Fig. 2]



Cu – Cuticle; UE – Upper epidermis; Pal – Palisade cells; Xy – Xylem; Ph – Phloem; LE – Lower epidermis; SP – Spongy paranchyma; CS-Crystal sheath; Co – Collenchyma; Pa-Parenchyma; Me-Mesophyll cells.

Fig 2: T.S. of *Carissa carandas (L.)* Leaf

Quantitative microscopy

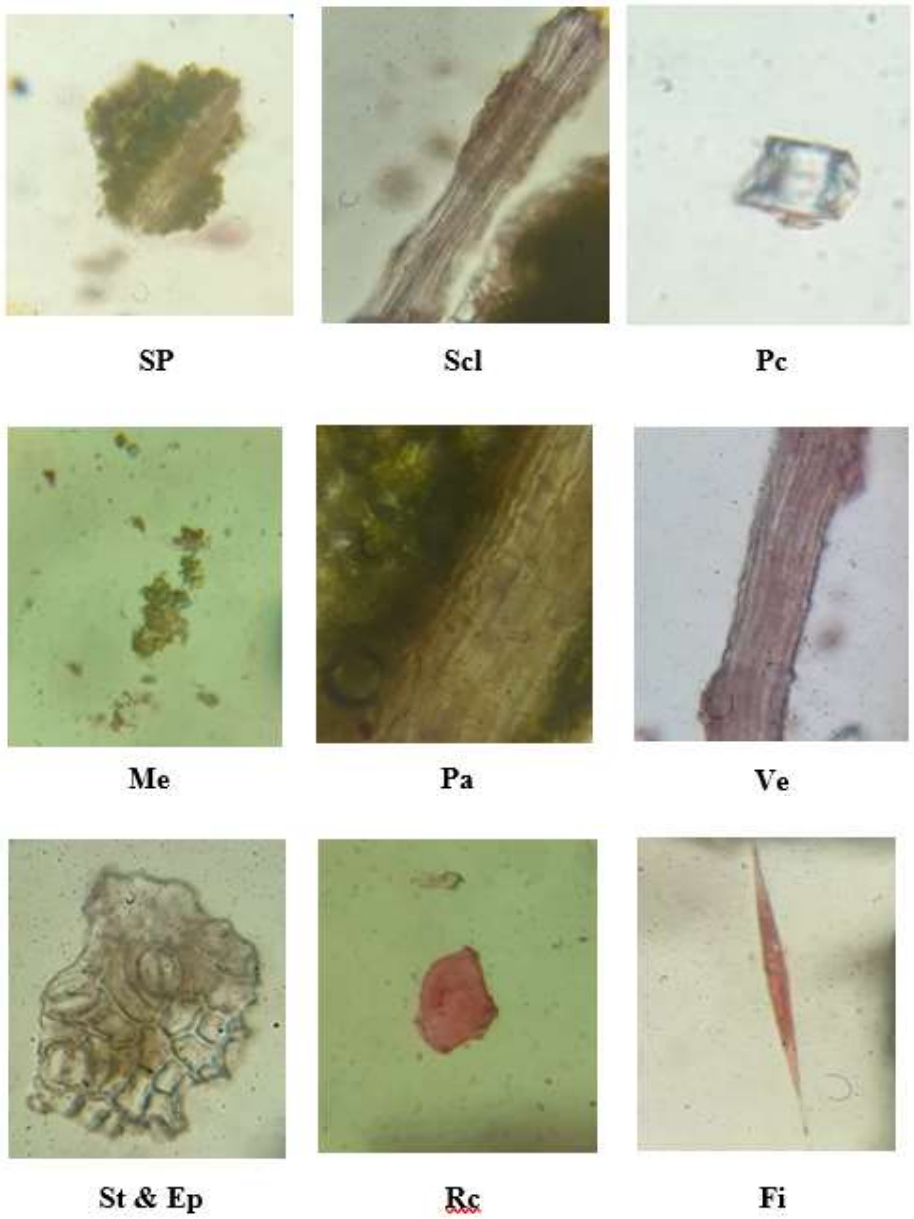
The quantitative measurements of stomatal number, stomatal index, palisade ratio, vein islet and veinlet termination numbers were determined andreported in Table 2.

Table 2: Quantitative microscopy of *Carissa carandas* (L.) leaf

S.NO	Parameters	Average (cells/mm^2)
1	Epidermal number – Lower	288
	- Upper	261
2	Stomatal number - Lower	135
3	Stomatal Index - Lower	34.09
4	Palisade Ratio	10 - 15
5	Vein islets	6
6	Vein termination	32

Powder microscopy

The powder microscopical evaluation of *Carissa carandas* (L.) leaves showed the presence of Spongy parenchyma cells, epidermal cells in surface view with anisocytic stomata, cluster view of mesophyll tissue; fibres, sclerenchyma cells, vessels, parenchyma cells and calcium crystals [Fig 3]



SP – Spongy paranchyma; Sc – Sclerenchyma; Pc – Prismatic crystals; Pa-Parenchyma; Me-Mesophyll tissues Ve-vessels; St & Ep – Stomata with epidermal cells; Rc - Rosatte crystal; Fi- Fibres

Fig 3: Powder microscopy of *Carissa carandas (L.)* leaf

Fluorescence analysis

Fluorescence analysis of *Carissa carandas* leaf powder with various solvents are given [Table 3].

Table 3: Determination of fluorescence analysis of *Carissa carandas* (L.)

SAMPLE + REAGENT	OBSERVATION		
	Visible (<400 nm)	UV short wave (265 nm)	UV long wave (365 nm)
Powder + HCl	Green	Black	Green
Powder + HCl + H_2O	Green	Black	Green
Powder +H_2SO_4	Red	Black	Dark Green
Powder +H_2SO_4 + H20	Brown	Black	Dark Green
Powder + HNO_3	Dark Orange	Black	Green
Powder + HNO_3+ H_2O	Orange	Brown	Light Green
Powder + 20% NaOH	Brown	Dark green	Green
Powder + AlcoholicNaOH	Green	Black	Green
Powder + Acetic acid	Green	Black	Light Green
Powder + Fe_3Cl_4	Green	Black	Dark Green
Powder + Iodine	Orange	Brown	Green
Powder + Picric Acid	Light Green	Dark Green	Fluorescent Green
Powder+ammonia	Red	Black	Dark Green
Powder+water	Dark Green	Black	Green

Physico chemical parameters

The extractive values of petroleum ether, ethyl acetate, ethanol and water extracts are given [Table 4].

Table 4: Determination of Physico-chemical parameters of *Carissa carandas* (L.)

S.NO	PHYSICO –CHEMICAL CONSTANT	REPORTS
1	Foreign Matter	NIL
2	Loss on Drying	3.46693 ± 0.18498% W/W
4	Total solid contents	96.533 % w/w
5	Bitterness Value	NIL
6	Volatile oil content	NIL
7	Petroleum ether extractive	0.177333 ± 0.070038 % w/w
8	Ethyl acetate extractive	0.186667 ± 0.069867 % w/w
9	Methanol extractive	0.448333 ± 0.068237 % w/w
10	Ethanol extractive	0.326000 ± 0.045826 % w/w
11	Aqueous extractive	0.364333 ± 0.05208 % w/w
12	Total ash	0.9215 ± 0.05809 % w/w
13	Water soluble ash	0.5812 ± 0.0416% w/w
14	Acid insoluble ash	0.04125 ± 0.00781%w/w
15	Presence of Inorganic elements	Chloride, Sulphate, Iron
16	Absence of Inorganic elements	Aluminium, Arsenic, Copper, Lead, Mercury, Magnesium, Silver

Determination of the phytochemical analysis of *Carissa carandas* (L.)

Table 5: Determination of the phytochemical analysis *Carissa carandas* (L.)

S.no	Phytochemical Analysis	Observation
1	Test for Carbohydrate	+
2	Test for Flavonoids	+
3	Test for Alkaloids	+
4	Test for Saponins	+
5	Test for Tannins	+
6	Test for Coumarin	+
7	Test for Quinone	+
8	Test for Glycoside	+
9	Test for Protein	-

10	Test for Gum and mucilage	-
11	Test for Phenolic compound	+
12	Test for Anthocyanin	-

('+'-presence, '-'Absence)

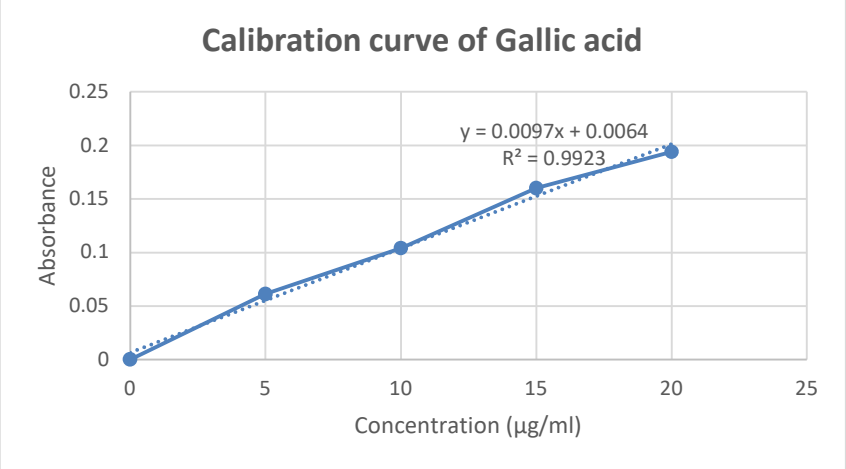
Quantitative estimation of phytoconstituents

Quantitative analysis such as total tannin content, total phenolic content and total flavonoid content were estimated for the HAECC.

Estimation of phenolic content

Table 6: Determination of Gallic acid Equivalent

S.NO	CONCENTRATION		ABSORBENCE	
	Gallic acid (µg/ml)	HAECC (µg/ml)	Gallic acid *Mean ± SEM	HAECC *Mean ± SEM
1	0	0	0	0
2	5	5	0.061 ±0.0008	0.054 ±0.0012
3	10	10	0.104 ±0.0010	0.106 ±0.0015
4	15	15	0.16 ±0.0028	0.145 ±0.0008
5	20	20	0.194 ±0.0012	0.1856 ±0.0012
			GAE	92.577 mg/g



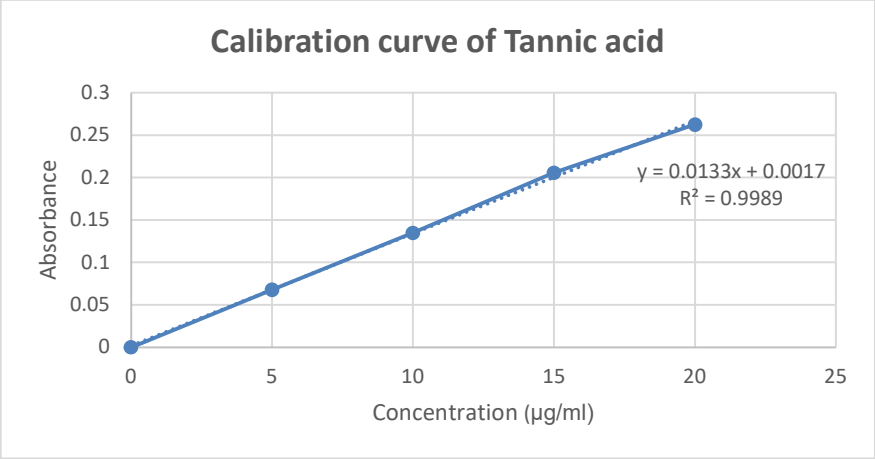
The Gallic acid equivalent in HAECC was found to be 92.577 mg/g.

Fig 4: Calibration curve of Gallic acid

Estimation of tannin content

Table 7: Determination of Tannic acid Equivalent

S.NO	CONCENTRATION		ABSORBENCE	
	Tannic acid (µg/ml)	HAECC (µg/ml)	Tannic acid *Mean ± SEM	HAECC *Mean ± SEM
1	0	0	0	0
2	5	5	0.068 ±0.0012	0.053 ±0.0009
3	10	10	0.135 ±0.00088	0.123 ±0.00120
4	15	15	0.2057 ±0.00033	0.194 ±0.00166
5	20	20	0.2627 ±0.00033	0.257 ±0.00208
			TAE	95.95 mg/g



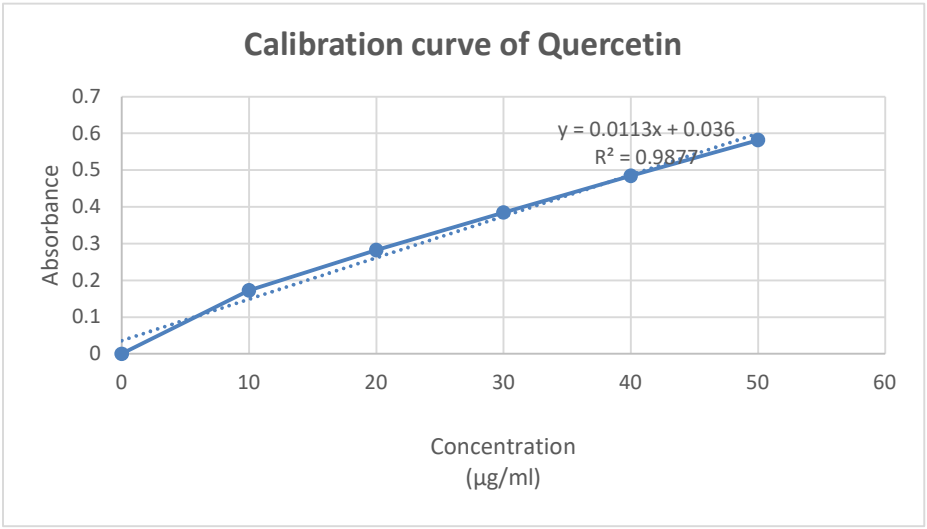
The Tannic acid equivalent in HAECC was found to be 95.95 mg/g.

Fig 5: Calibration curve of Tannic acid

Estimation of flavanoid content

Table 8: Determination of Quercetin Equivalent

S.NO	CONCENTRATION		ABSORBENCE	
	Quercetin (µg/ml)	HAECC (µg/ml)	Quercetin *Mean ± SEM	HAECC *Mean ± SEM
1	0	0	0	0
2	10	10	0.173 ±0.00088	0.1527 ±0.00088
3	20	20	0.2827 ±0.00033	0.2033 ±0.00055
4	30	30	0.3847 ±0.00033	0.3577 ±0.00426
5	40	40	0.4847 ±0.00067	0.4707 ±0.00219
6	50	50	0.5816 ±0.00033	0.5738 ±0.00246
			QE	95.04 mg/g



The Quercetin equivalent in HAECC was found to be 95.05 mg/g

Fig 6: Calibration curve of Quercetin

Phenetics

The results of Phenetics obtained is in in Table 6,7 & Fig 7 ,8.

Table 9: Table of similarity of *Carissa carandas* with its four other species

	C. carandas	*C. congesta*	*C. opaca*	*C. inermis*	*C. spiranum*
C. carandas	1	1	1	1	1
C. congesta	0	1	0	1	1
C. opaca	0	0	0	0	1
C. inermis	0	1	0	0	1
C. spiranum	0	1	1	1	1

Leaf shape : Ovate - lanceolate =1, not ovate - lanceolate =0
Leaf size : Small = 1, not small = 0
Leaf apex : obtuse = 1, not obtuse = 0
Leaf petiole : Grooved = 1, not grooved = 0
Leaf base : Rounded or Cunate = 1, not rounded or cunate =0

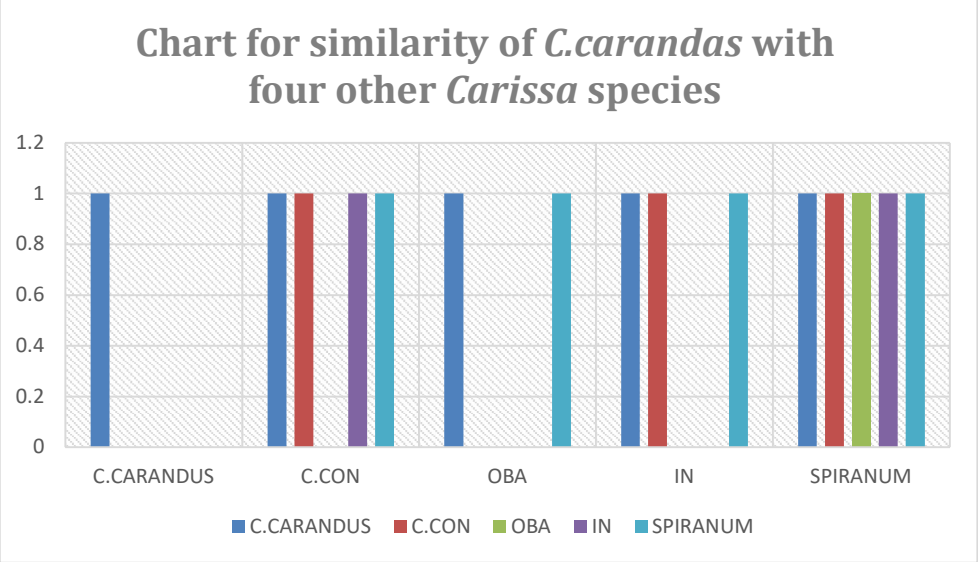


Fig 7: Chart of similarity of *C.carandas* with its four other species

Table 10: Table of dissimilarity of *Carissa carandas* with its four other species

	C. carandas	*C. congesta*	*C. opaca*	*C. inermis*	*C. spiranum*
C. carandas	1	0.6	0.2	0.4	0.8
C. congesta	0.6	1	0.6	0.8	0.8
C. opaca	0.2	0.6	1	0.8	0.4
C. inermis	0.4	0.8	0.8	1	0.6
C. spiranum	0.8	0.8	0.4	0.6	1

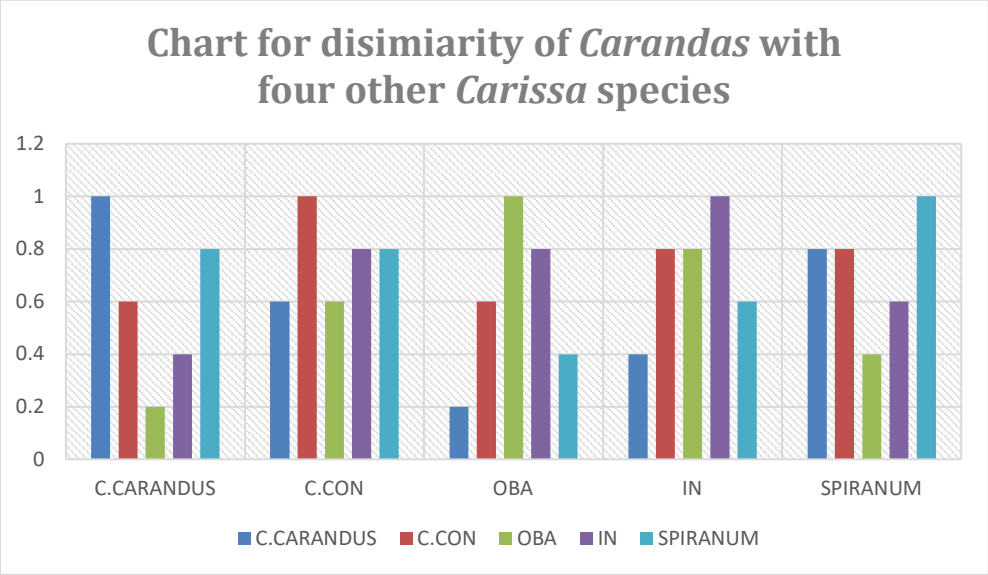


Fig 8: Chart of dissimilarity of *C.carandas* with its four other species

Sample matching coefficient SSM = NS / NS + ND X 100
The matching co-efficient of *Carissa carandas* with respect to other species was found to be 60%.

Key characters of *Carissa carandas*

- shape of the leaf is ovate - lanceolate
- size of the leaf is relatively smaller compared to *C.inermis*
- The apex of the leaf is obtuse
- The petiole of the leaf is grooved
- The base of the leaf is rounded or cunate

CONCLUSION

This study provides a comprehensive pharmacognostical evaluation of *Carissa carandas (L.)* leaves, highlighting important diagnostic features that contribute to its identification and standardization for medicinal use. The detailed examination of macroscopic and microscopic characteristics, along with quantitative leaf parameters, confirms the presence of key anatomical markers such as anisocytic stomata, reticulate venation, and the occurrence of calcium oxalate crystals. The results, including phenetic analysis and phytochemical screening, offer a reliable reference for quality control and support the traditional medicinal applications of *Carissa carandas*. These findings lay the foundation for further pharmacological and clinical investigations to validate and expand the therapeutic potential of this species.

CONFLICT OF INTEREST
Conflict of interest declared none.

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No funding was obtained from any source.

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