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PHARMACOGNOSTICAL STUDIES OF *Carissa carandas* Linn Leaves

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ABSTRACT

Carissa carandas Linn (Apocynaceae) is a thorny evergreen shrub known as Karonda. It has small berry-shaped fruits added as a flavoring to numerous pickles and spices in northern India. The study's objective is to analyze the Pharmacognostical standards of the leaves of *C. carandas*. Pharmacognostical standards were established using techniques such as macroscopy, microscopy, physicochemical parameters, extractive values, fluorescence analysis, and phytochemical screening. The latest findings may be a valuable resource for knowledge and could offer appropriate standards for identifying this plant material in upcoming research and application.

Keywords: *Carissa carandas*, Fluorescence, Macroscopy, Microscopy, Physicochemical

1. INTRODUCTION

Carissa carandas is a thorny evergreen shrub belonging to the Apocynaceae family, also referred to as Karonda. It has tiny, berry-like fruits that are added to various pickles and used as a spice in northern India **[1]**. The plant is also known as Kilaakkaai in Tamil, Karonda in Devanagari, Vakkay in Telugu, Karamardaka in Sanskrit, Koromcha in Bengali, Bengal currant or Christ's thorn in English, and Karja tenga in Bengali (Assam), Cu huang guo, Tz-huang-kuo in Chinese **[2]**, etc.

Table 1: Taxonomical classification

Class	**Angiosperms**
Sub-class	**Eudiots**
Superorder	**Asterids**
Order	**Gentianales**
Family	**Apocynaceae**
Genus	***Carissa***
Species	***carandas***

Karonda is a fruit native to dry climates with a reasonable quantity of vitamin C and minerals. The Karonda fruit helps treat anemia and is an astringent, antiscorbutic medicine for biliousness **[3]**. A Bitter and anthelmintic roots. Fruits that are still green have astringent and antiscorbutic properties. When remittent fever first appears, leaves are brewed into a decoction and administered. According to reports, the bark extract is cardiotonic **[4]**. The plant is integral to several ayurvedic formulations and preparations, including Kalkantaka rasa, Kshudra karvanda yoga, Hridya mahakashaya, Marma gutika, and Marichadi vati **[5]**. An analysis of the root extract's phytochemical composition revealed that it was primarily composed of cardiac glycosides, triterpenoids, phenolic compounds, tannins, and moderate levels of alkaloids, flavonoids, and saponins **[6]**. The current study examines different pharmacognostic criteria, including macroscopic, microscopic, physicochemical, fluorescence, and phytochemical analyses of the plant.

2. METHODS AND MATERIALS

2.1 Plant material

In March, the *Carissa carandas* plant was collected from the Thirukkalukundram, Chengalpet district, Tamilnadu, India. Dr. Sunil Kumar, a taxonomist at the Siddha Central Research Institute in Arumbakkam, India, authenticated the plant. A voucher specimen (343.19082201) was on display in the departmental museum's herbarium section for future use.

2.2 Pharmacognostical study

2.2.1 Macroscopy

Macroscopic analysis of fruits, such as form, shape, color, odor, taste, and other parameters, were observed and studied as per the method.

2.2.2 Microscopy

The sample was kept in a fixative Federal Aviation Administration for 48 hours. A sharp blade cut the preserved specimens into thin transverse pieces, and the sections were dyed with safranin. Under bright field light, transverse sections were captured using an Axiolab-5 trinocular microscope connected with an axiocam 208 color digital camera **[7]**. A scale bar displayed the magnifications.

2.2.3 Quantitative microscopy

The fresh leaf samples were boiled with chloral hydrate solution and stained with safranin; slides were prepared with a drop of 50% glycerol **[8]**. Epidermal number, Vein termination, Vein islets, Stomatal number, Stomatal index, and Palisade ratio were observed using Olympus BX43 trinocular microscope attached to a drawing tube and digital camera under a bright field.

2.2.4 Powder microscopy

After being cleared with 0.1% chloral hydrate, a little amount of the powdered sample was placed on a microscopic slide with a drop of 50% glycerol **[9]**. Characters were examined using a bright field and polarised light source with an Axiolab-5 trinocular microscope equipped with an Axiocam 208 color digital camera. Diagnostic characteristics were photographed and recorded as photomicrographs.

2.3 Physico-chemical analysis

2.3.1 Ash values

Ash values are important parameters for evaluating the efficacy and purity of herbal medications. Total ash values were calculated by gradually heating the powdered sample between 100°C and 200°C until it turned white. The resulting powder was then dried and weighed **[10]**. Acid insoluble ash values was calculated by dissolving a part of the total ash in Conc. HCL, collecting the solution, washing it on filter paper, cooling it in a desiccator, and weighing it. Water soluble ash was also determined similarly.

2.3.2 Extractive values

5g of sample were macerated with various polarity solvents and agitated on an orbital shaker for 8 hours to obtain the extractive values. Filter, dried-off, and weighed extracts were used **[11]**. Extractive values were determined and calculated.

$$\text{\% Extractive value of solvent} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

2.3.3 Loss on drying (LOD)

The values were determined by drying the material (10 g) in a moisture content balance and set temperature between 85–100°C. Drying was maintained till there was no difference between the two subsequent weight readings of more than 5 mg **[12]**.

2.3.4 Fluorescence analysis

The fluorescence analysis can be held with dried leaf powder treated with different chemical reagents such as Iodine, Ferric chloride, Ethanol, Acetic acid, NaOH, Water, and Ammonia under daylight, short-wavelength, and long-wavelength to analyze the fluorescence behavior of powdered leaf **[13]**.

2.4 Preliminary phytochemical screening

The five different extracts were subjected to a preliminary phytochemical study. The leaf extracts are Pet ether, Chloroform, Ethyl acetate, ethanol, and methanol of *C. carandas* were performed for the presence of different chemical components per the standard procedure **[14]**.

3. RESULT AND DISCUSSION

3.1 Macroscopy

The leaves are simple, opposite, oval, and green in color, with a glossy look on the upper surface and a duller green shade on the lower surface; the edge is entire with the short petiole **(Figure 1)**, and they lack any distinct aromas or tastes.

3.2 Microscopy

Petiole: TS is oval in shape with two-minute protuberances at the upper region. The outermost single-layer epidermis is covered by thick cuticles and few unicellular covering trichomes; the cortex is made up of eight to nine layers of collenchyma cells; few rosette crystals are seen; a discontinuous crystal sheath is seen surrounding the stellar tissue, which is composed of outer phloem embedded with few crystals in continuation with xylem elements arranged in the form of an arc **(Figure 2)**.

TS of leaf Midrib

The TS of a leaf through midrib shows distinct layered epidermis covered by a distinct cuticle followed by single-layered hypodermis; two to three layers of collenchyma cells followed by two layers of parenchyma is present below; a one layer of discontinuous crystal sheath is present as an arch below the phloem of centrally placed collateral vascular bundle; few prismatic and rosette crystals in the phloem region and few starch grains are seen randomly distributed **(Figure 3)**.

Lamina: The TS of the lamina is dorsiventral and hypostomatic; distinct layered wavy epidermis coated by thick cuticle and few covering trichomes; mesophyll is made up of outer two layers of radially elongated palisade parenchyma cells followed by 3 to 4 layers of spongy parenchyma, veins are seen traversing through the mesophyll tissue.

3.3 Quantitative microscopy

The quantitative parameters discovered through the microscopic study of leaf epidermal peelings were recorded in **Table 2**. The leaves have anisocytic stomata on their lower surface and are hypostomatic; **(Figure 4)**.

3.4 Powder microscopy

The powder is dull green with a slightly bitter taste and no characteristic odor. It shows uniseriate trichomes, fragments of the epidermis with anisocytic stomata, mesophyll with crystals, spiral vessels, crystal fiber, prismatic, and cluster crystals **(Figure 5)**.

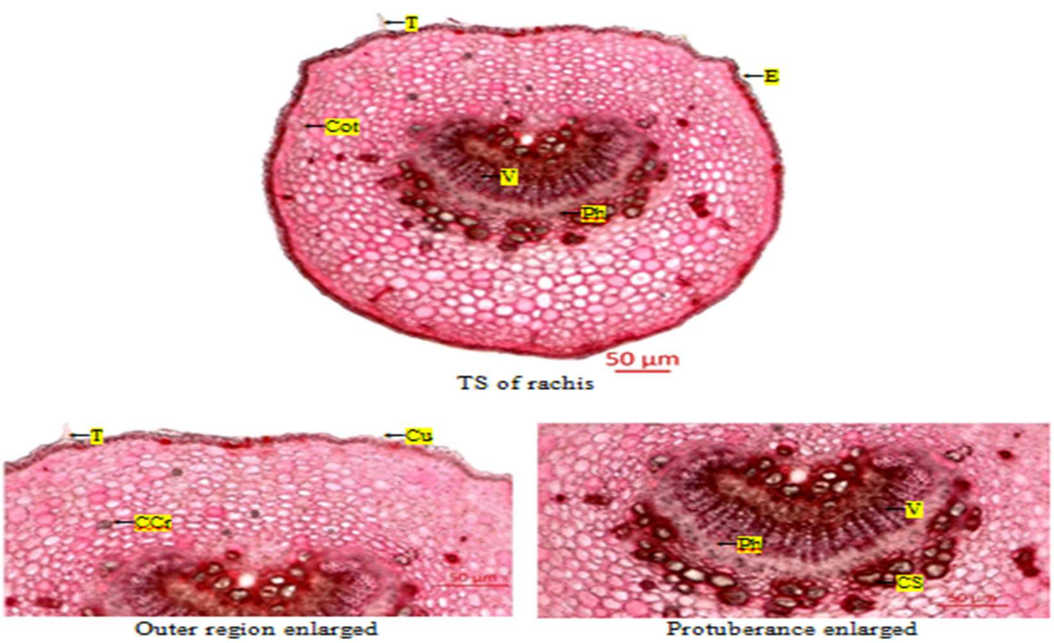
3.5 Preliminary phytochemical screening

The extracts were subjected to qualitative analysis of the leaf of *C. carandas* for the identification of phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, terpenoids, carbohydrates, and amino acids etc. **(Table 3)**.

3.6 Physicochemical parameter

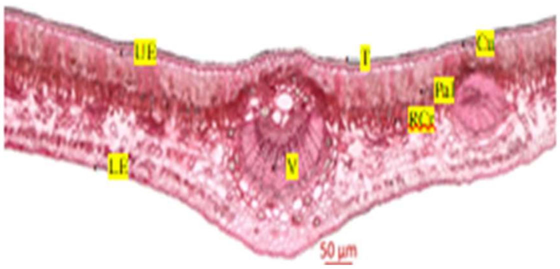
Physicochemical analysis of leaf powder was subjected to Loss on drying, ash value, and extractive value are presented in **Table 4**. The fluorescence analysis of *Carissa carandas* leaf under daylight and UV (short (254nm), long (356nm)) light is recorded in **Table 5**.

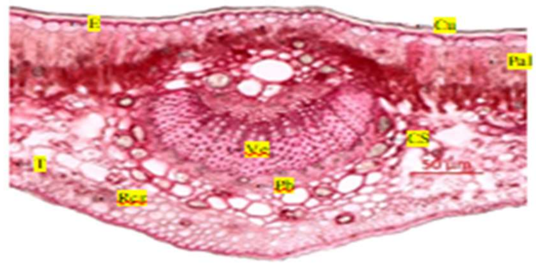
Figure 1: *Carissa carandas* twig with leaves



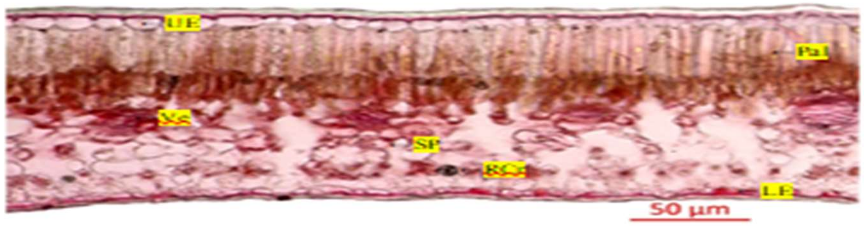
CCr- Cluster crystal; Cot – Cortex; Cu – Cuticle; E – Epidermis; Ph – Phloem; T – Trichome; V – Vessel

Figure 2: TS of *Carissa carandas* petiole





TS lamina through midrib **Middle region enlarged.**



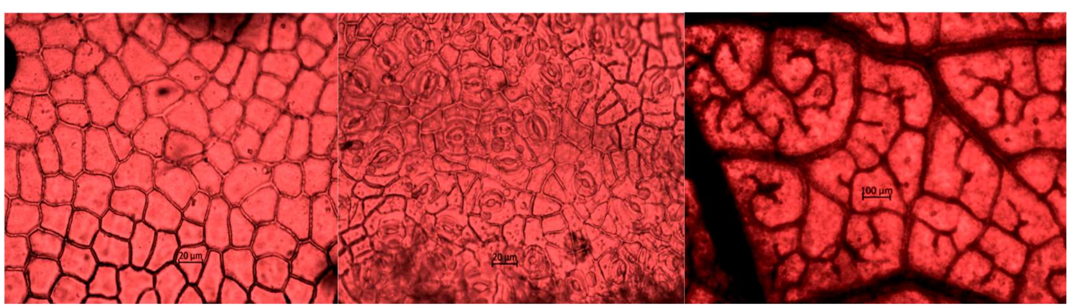
Lamina region enlarged

CS - Crystal sheath; Cu - Cuticle; E - Epidermis; LE - Lower epidermis; Pa - Parenchyma; Pal - Palisade; Rap - Raphide; Ph - Phloem; UE - Upper epidermis; Ve – Vein

Figure 3: TS of *Carissa carandas* leaves

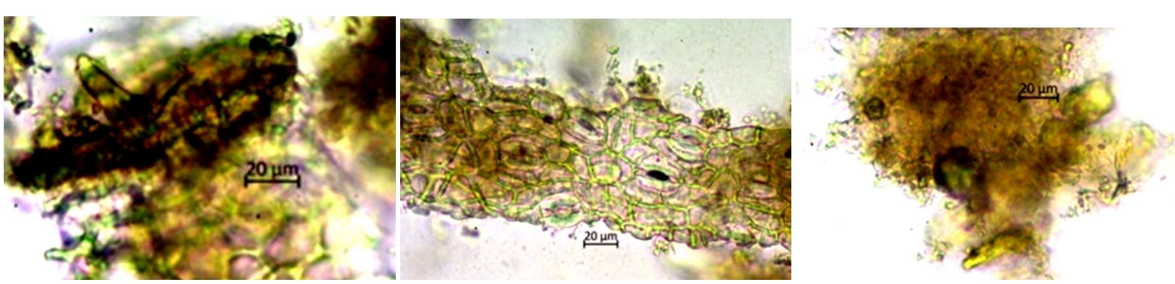
Table 2: Quantitative microscopy of *Carissa carandas* leaf

Parameters	Upper (/mm^2)	Lower (/mm^2)
Epidermal number	260- 270	290 – 340
Stomatal number	-	140 – 160
Stomatal index	-	5 – 7
Palisade ratio	24 – 30	
Vein islets number	4 – 6	
Vein termination number	34 – 44	

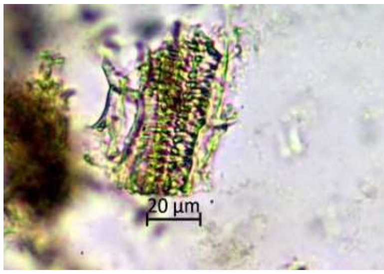


Upper epidermis **Lower epidermis** **Vein islet and vein termination**

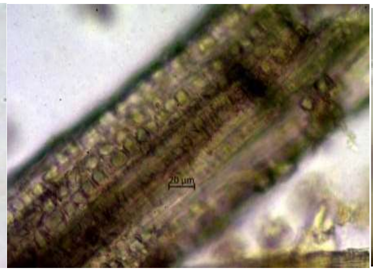
Figure 4: Quantitative microscopy of *Carissa carandas* leaf



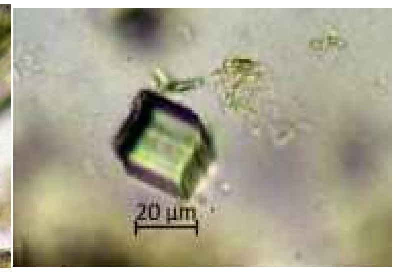
Simple trichome **Epidermis with anisocytic stomata** **Mesophyll fragment**



Spiral vessels



Crystal fiber



Prismatic crystals

Cluster Crystal

Figure 5: Powder microscopy of *Carissa carandas* leaf

Table 3: Preliminary phytochemical screening of *C.carandas* leaves

S. No.	Secondary metabolite	Pet. ether	$CHcl_3$	Ethyl acetate	Ethanol	Methanol
1.	Carbohydrate	-	+	+	+	+
2.	Protein	+	-	+	+	+
3.	Amino acid	-	-	+	+	+
4.	Alkaloid	+	+	+	+	+
5.	Flavonoid	+	-	-	+	+
6.	Glycosides	-	-	+	+	+
7.	Tannins	-	-	+	+	+
8.	Saponin	+	+	_	+	+
9.	Terpenoids	+	+	+	+	+
10.	Sterols	+	+	+	+	+
11.	Fats and oil	+	+	-	-	-
12.	Gum and mucilage	-	-	-	-	-

Table 4: Physicochemical parameters of the leaf of *Carissa carandas*

S. No.	Parameters	Values% w/w
1.	Ash values	
1.1	Total ash	20
1.2	Acid insoluble ash	17.5
1.3	Water soluble ash	16.3
2.	Extractive values	
2.1	Pet ether	5.6
2.2	Chloroform	8.3
2.3	Ethyl acetate	4.5
2.4	Ethanol	11.7
2.5	Methanol	13.2
3.	Loss on drying	15

Table 5: Fluorescence analysis of the leaf of *Carissa carandas*

S. No.	Reagents	Visible light	UV- 254nm	UV- 356nm
1.	Powder	Light green	Dark green	Black
2.	Powder + Iodine	Yellow	Brown	Brown-green
3.	Powder + $FeCl_3$	Brown	Green	Black
4.	Powder + Ethanol	Fade green	Green	Black
5.	Powder + Acetic acid	Light green	Green	Dark Green
6.	Powder + NaOH	Mustard	Green	Black
7.	Powder + Water	Light brown	Green	Black
8.	Powder + Ammonia	Light green	Blackish green	Black

DISCUSSION

Standardization of a crude drug is a crucial component of determining authenticity concerning purity and quality. It encompasses a variety of factors, including botanical, physical, chemical, and biological ones. Organoleptic and histochemical evaluations are among these variables, and they are regarded as a vital part and initial method for ensuring the proper identification of herbal medications.

Traditional herbal therapy uses *C. carandas* (Apocynaceae) extensively to treat various ailments. Appropriately identifying and verifying a plant's leaf is proven to benefit from such research of pharmacopeial standards. The macroscopical features disclosed the leaf's color, flavor, texture, and kind. According to microscopical analyses, the cuticle layer, epidermis, parenchyma cells, and mesocarp cells were all present. The presence of vascular bundles, Sclereids, and calcium oxalate crystals can identify leaf powder. Physical characteristics used in this investigation included ash value, moisture content, and extractive values.

A preliminary phytochemical analysis determined whether the plant's leaves contained any components. Alkaloids, saponin, glycosides, flavonoids, tannins, and phenolic substances are present, along with lipids, oils, volatile oils, carbohydrates, gums, and mucilage. The fluorescence analysis of the leaf powder revealed that it was light green in the daylight and dark green and black in the short and long wavelengths.

Standardization is necessary. This help creates identification and quality profiles that may be applied to the overall quality assurance and safety monitoring of herbal medications. Therefore, creating a Pharmacognostical standard is essential. The findings of the current study are promising and can be utilized as useful reference data for Carissa carandas Linn leaf uniformity.

CONCLUSION

Carissa carandas leaves were used to establish various Pharmacognostical characteristics, including microscopic, macroscopic, physiochemical, and fluorescence parameters and phytochemical analysis. This herbal medicine's quality, safety, and effectiveness can be ensured using these results as adequate quality control procedures. This work is a significant first step, and further research in the fields of pharmacology and phytochemistry is needed.

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