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Pharmacognostic Characterization And Multi-Target Pharmacological Evaluation Of Carissa Carandas Leaves

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ABSTRACT

The present study investigates the pharmacognostic, phytochemical, and pharmacological potential of *Carissa carandas* leaves. The research involves the collection, authentication, and systematic evaluation of the plant material. Macroscopic and microscopic characteristics were studied to ensure proper identification, while physicochemical parameters such as total ash value, extractive values, moisture content, and pH determination were analyzed. Thin Layer Chromatography (TLC) was employed for fingerprint profiling, and fluorescence analysis was conducted to assess chemical characteristics.

Preliminary phytochemical screening was performed to detect major secondary metabolites qualitatively and quantitatively. Total phenolic and flavonoid content was estimated, as these compounds contribute to antioxidant activity. The antioxidant potential of the extracts was assessed using the DPPH radical scavenging method. Pharmacological evaluation included in-vivo anti-inflammatory activity using carrageenan-induced paw edema and cotton pellet-induced granuloma models.

The study aims to isolate and characterize bioactive compounds from *Carissa carandas* leaves, correlate their phytochemical composition with pharmacological activities, and contribute to scientific understanding and therapeutic applications. The findings may support the potential use of *Carissa carandas* in developing novel antioxidant and anti-inflammatory agents.

Keywords: *Carissa carandas*, phytochemical screening, antioxidant activity, anti-inflammatory activity, analgesic activity, TLC, pharmacognostic evaluation

1.0 INTRODUCTION

1.1 Inflammation

Swelling and redness are common signs of inflammation, which occurs when an illness or damage occurs (Chaudhari et al., 2013). Inflammation is an organism's defence mechanism against harmful stimuli and the beginning of tissue healing (Denko et al., 1992). If you have a condition like rheumatoid arthritis, for example, inflammation might be a contributing factor in the development of other health issues, such as rhinorrhea (Henson 1989). Assess the key underlying mechanisms is crucial to completely comprehend the inflammatory process: enzyme activation, inflammatory mediator synthesis, cellular movement and movement of fluid. (Vane and colleagues, 1995) As a result of protein denaturation, increased vascular permeability, and membrane alteration, it results in swelling, pain, fever, and redness in the affected area (Umapathy et al., 2010).

The people's biggest concern is that existing medications can induce toxicity and recurrence if they are stopped abruptly. As a result, the development of innovative anti-inflammatory medications that are both safe and effective is of great interest. Inflammation is mediated by a variety of cells, including those that produce histamine, serotonin, plasma kinins, prostaglandins, and lymphokines (Ghlichloo 2025).

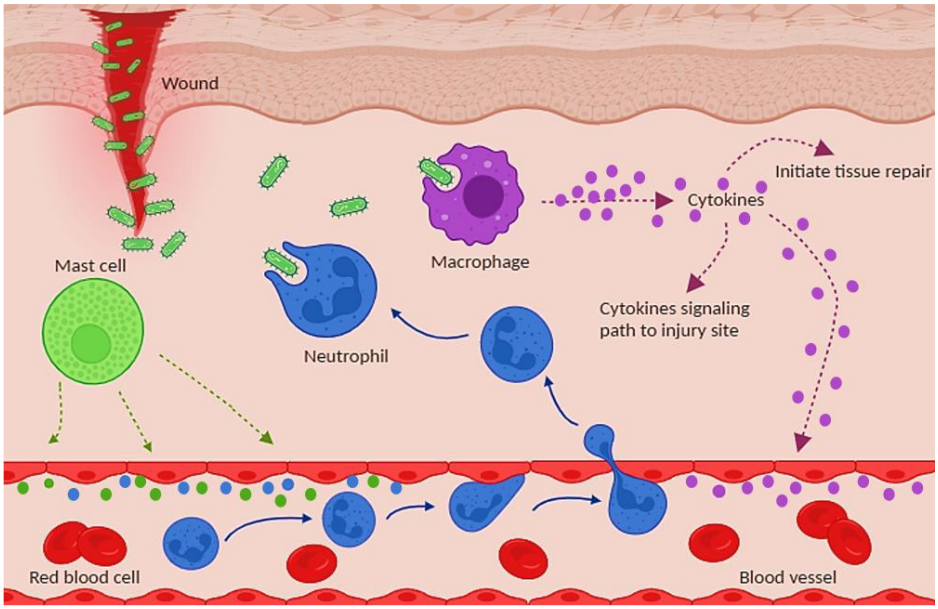


Figure 1.1 Schematic representation of the inflammatory process

1.2 Different types of Inflammation

1.2.1 Acute Inflammation

Sudden as well as acute inflammation, trauma or harmful substances, or tissue damage caused by microbial invasion can all result in acute inflammation. For instance, acute pneumonia or cellulitis both start out mildly, worsen swiftly, and can leave patients with lingering symptoms. Between acute and chronic inflammation, subacute inflammation can last for two to six weeks (Chen et al., 2017).

1.2.2 Chronic Inflammation

Chronic inflammation, which can last for a few months to years, is also known as slow, long-term inflammation. The cause of the injury, as well as the body's capacity to heal and overcome the damage, are the main determinants of the intensity and impact of chronic inflammation. One of the most prevalent conditions involving these mediators is rheumatoid arthritis, in which ongoing inflammation results in pain and the breakdown of bone mediators, which can cause serious disability and changes that can lessen it (Jo et al., 2010).

1.3 Inflammation causes

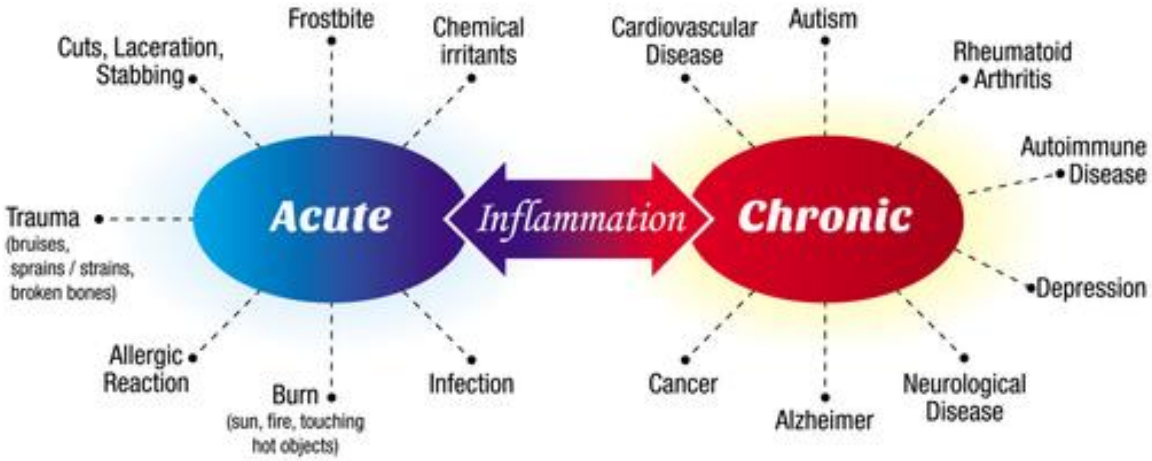


Figure 1.2 Causes of inflammation

1.4 Inflammatory Mediators and their Biomarkers

With discovery of several cellular and molecular mediators of inflmmation, as well as the creation of sensitive biomarkers, our knowledge of inflammation and how it contributes to disease progression has grown exponentially.

These biomarkers include:

- Nitrogen oxide species (NOS) and reactive oxygen species (ROS)
- DNA adduct formation

- Chemokines and cytokines and (IL-6; TNF-α)
- Prostaglandins
- Proteins
- COX
- Growth factors as well as transposable elements connected to inflammatory response (NF-kappa) (Stone et al., 2022).

1.5 Analgesics Mechanism of Action:

NSAIDs are historically thought to have analgesic effects because they inhibit the formation of prostaglandin-producing enzymes. A variety of peripheral and central processes, in addition to the peripheral reduction of prostaglandin production, are used by NSAIDs to provide analgesic effects. It is now understood that the COX enzyme present in COX-1 and 2 complex characteristic forms. In contrast to COX-2, elevated in inflammatory cells, COX-1 is a naturally occurring enzyme in healthy tissues. Arresing of COX-2 enzyme potency is the widely likely action action for NSAID-based analgesia, and a risk of side effects should depend on how much COX-1 is inhibited relative to COX-2.

1.6 Plant as Anti-Inflammatory, Analgesic, and Antipyretic Drug Sources

Since hundreds of centuries all across the entire planet, traditional healthcare approaches have been based on the basis of plants and their metabolites, and they are being employed for medical purposes as they give new treatments. Plants have a variety of active chemicals that may be isolated, identified, and quantified, allowing for the creation of new medications to treat inflammation (Ahmed et al**.**, 2011). Willow tree bark has been used as an ibuprofen and hypotensive since 400 BC, which revealed the presence of aspirin, which was first used as a potent medication in the treatment of rheumatic illnesses in 1899**.** According to estimates, almost 40% of all therapeutics available today come either implicitly or explicitly from biological compounds, with plants accounting for 25%, microbes for 13%, and animals accounting for 3%. Approximately, 140,000 secondary metabolites, mostly from higher plants, have been extracted and chemically characterized. Despite this development, the bulk of naturally occurring compounds is still completely unknown in the term,s of their pharmacological function (Gautam et al., 2009).

1.7 Herbal Medicine and Natural Products in Inflammation and Pain Management

Herbal medicine has been an integral part of traditional healing systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani, for centuries. Various medicinal plants exhibit potent anti-inflammatory and analgesic properties due to the presence of bioactive compounds such as flavonoids, alkaloids, terpenoids, tannins, and polyphenols. These phytochemicals modulate inflammatory pathways, including the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, suppression of pro-inflammatory cytokines, and scavenging of reactive oxygen species (ROS). Herbs such as *Curcuma longa* (turmeric), *Zingiber officinale* (ginger), *Boswellia serrata* (frankincense), and *Withania somnifera* (ashwagandha) have been extensively studied for their anti-inflammatory and analgesic effects (Leonti et al., 2013).

2.0 MATERIAL AND METHODS

Collection and authentication of the selected plant materials from taxonomic division of Banaras Hindu University (BHU), Varanasi. A voucher specimen was deposited for future reference.

2.1 Pharmacogenetic studies

2.1.1 Morphological studies

For morphological observations, fresh leaves and roots of both plants were used. The macromorphological features of leaves and roots were observed under the magnifying lens (Tyler et al., 1977).

2.1.2Microscopical evaluation

The morphological characteristics of the specimen (leaves and bark) were studied and the photographs were taken. For microscopic studies, the transverse section (TS) was preferred over the longitudinal section. The fine sections of leaves were cut by free hand. The chlorophyll and the other pigments of the plant were removed by treating the sections with 5% potassium hydroxide and 20% chloral hydrate. Photographs of different magnifications were taken with Olympus Microscope, Model Olympus (India), attached to YOKO CCD Camera (Khandelwal et al., 2008;).

2.2 Extraction

Extraction was done according to standard procedure using analytical grade solvents. Successive solvent extraction was carried out by using solvents according to the nature of secondary metabolites. The coarse powder of the dried plant material was extracted by using petroleum ether, methanol, and water (non-polar to polar) successively by the Soxhlet extractor.

2.3 Physicochemical evaluation
Various physicochemical parameters were carried out such as determination of total ash, acid insoluble ash value, water-soluble ash values, extractive values, moisture content, and foreign particles, etc. as per WHO guidelines (Vaghasiya 2008).

2.4 Determination of loss on drying
Two grams of dried crude powder were taken in an evaporating dish and then dried in an oven at 105 ºC till constant weight was obtained. The weight was noted after drying was noted and after drying loss on drying was calculated. The percentage loss on drying was calculated based on initial weight.

2.5 Determination of total ash
Two grams of dried powder of crude drug were taken in a silica crucible and heated, gradually increasing the heat to 450 °C, until it was white, indicating the absence of carbon. Ash was cooled in desiccators and weighed without delay. Total Ash value was calculated as mg/g of air-dried material.

2.6 Determination of acid-insoluble ash
25 ml of hydrochloric acid (70 g/L) was added to the crucible containing total ash. It was covered with a watch glass and heated gently for 5 min to boil. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash-less filter paper and it was washed with hot water until the filter was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and heated till constant weight was obtained. The residue was allowed to cool in a desiccator for 30 min and then weighed without delay. Acid insoluble ash was calculated as mg/g of air-dried material.

2.7 Determination of water-soluble ash
To the crucible containing the total ash, 25 ml of water was added and boiled for 5 min. The insoluble matter was collected on ashless filter paper. It was washed with hot water and heated in a crucible for 15 min. The weight of insoluble matter was subtracted from the weight of total ash. The content of water-soluble ash was calculated as mg/g of air-dried material.

2.8 Determination of petroleum ether soluble extractive value
Five grams of dried powder of Crude drugwas taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105 °C till constant weight was obtained. The percentage of the extractable matter was calculated concerning the sample taken initially.

2.9 Determination of methanol soluble extractive value
Five grams of dried powder of Crude drugwas taken in 100 ml of methanol in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105 °C till constant weight was obtained. The percentage of the extractable matter was calculated with reference to the sample taken initially.

2.10 Determination of water-soluble extractive value
Five grams of dried powder of Crude drugwas taken in 100 ml of water in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105 °C till constant weight was obtained. The percentage of the extractable matter was calculated with reference to the sample taken initially.

2.11 Solubility
The quantitative solubility test of methanolic extract was determined in different solvents. Five mg of extract was weighed for the solubility test for different solvents. The extract was added to each solvent till it was saturated. The solubility was calculated in mg/ml.

2.12 Plant Extract Preparation
The collected (2.5 kg) plant materialswere air dried and followed by tray dryer under favourable condition and powdered it. The powdered crude drug (1.2 kg) was extract out with PE, Methanol andwater by Soxhlet extractor. The crude solution was further filtered and make syrupy mass by reduced pressure at 40°C and kept in desiccator.

2.13 Preliminary phytochemical screening
The chemical tests were performed for the detection of diverse secondary metabolites like alkaloids, glycosides, flavonoids, Steroids, tannins. The chemical concentration was determined by using the standard phytochemical tests. The plant extracts and was assessed for the existence of the phytochemical analysis by using the following standard methods (Debiyi et al., 1978; Roopashree et al., 2008; Sofowora 1993).

2.14 Thin Layer Chromatography

2.14.1 Procedure

The activated Utilized and activated comprised chromatographic plates made in a lab (Santiago et al., 2013).

2.14.2Preparation of sample

Before testing, 0.5 gm of extract (*Carissa carandas* leaves) were mixed with 5ml of C_2H_5OH and stirred for 3-30minutes by shaking. Centrifugation was used to remove insoluble particles, and the solution was filtered with filter paper.

2.14.3 Apparatus

A rack must have been utilised to hold the manufactured plates (typically 10 plates with predetermined spacings) as during drying process but rather for transit and storage. The glass plates must be 15-20 cm long, wide, and uniformly thick on the entire surface to hold the necessary amount of test samples, and the rack had to be compact enough to fit inside a hot - air oven. Preparation for use included immersing plates in an appropriate cleaning solution, rinsing until no visible water stains or oil spots were left, and then drying them thoroughly. When the coating substance was applied, there was no trace of line or dust on the plates.

2.15 Method

Preparation of the adsorbent

The coating material and water slurry was applied to the cleaning plates with thickness 0.25mm layer by the spreading apparatus. To activate the TLC plates, they were heated to 110°C for 30 minutes and then cooled. Initial air drying of coated plates was performed. Both the roughness of the coating and its homogeneity were examined by using reflected and transmitted light, respectively.

Chromatography in a saturated chamber

A saturated chamber is used for chromatography. Filter paper was used to line at least half of the chamber's interior walls, and mobile phase was added to the chamber until the filter paper was completely saturated and a 5mm thick layer formed. At the very least, the compartment was left at room temperature for an hour after it had been sealed up. A relative humidity of 50-60% was used for all procedures involving air exposure of the plate.

Test and Standard solutions

By micropipette, extract (*Allamanda blanchetii*) solution was applied to the beginning line, which was similar to approximately 15mm beyond the lower portion. There were 15mm-wide spots 15mm apart on the plate's edges, with a diameter no larger than 4mm. The starting line and mobile phase distances were measured. The test protocol specifies that the mobile phase should rise to a height of 10-15 centimetres.

Preparation of Mobile Phase

For proper separation of components, other mobile phase compositions were explored, including n-hexane: ethyl acetate: formic acid: acetic acid (7:3:0.1:0.1), and Methanol: water (7.7:2.3), Ethanol: acetone (2.5:7.5).

Development of chromatogram

The spots were left to dry. The plate was placed into the chamber as nearly vertically as feasible, utilising the platform's points just above mobile phase. The door to the chamber was closed. The chromatogram was made at room temperature, which allowed the solvent to rise the required amount. The Mobile phase was let to concentrate at normal temperature after the plate was taken off and the solvent front position was marked.

Chromatograms observation and interpretation

Short-wave and long-wave ultraviolet radiation were used to create the dots, which were subsequently viewed in daylight. A needle was used to mark the center of each area. The distance between the middle of every spot and site was measured and recorded, and the wavelength under which each spot was noticed was specified for each spot.

Rf value was computed as the ratio between the distance a specific component travelled on the adsorbent and the extent the solvent's leading edge travelled.

Whereby there is the distance between the application point and the centre of the substance under examination, and b is the height in between beginning of the implementation and the front of the solvents.

2.16 Total phenolic content

Total Phenolic content of Methanolic extract was determined employing the method involving Folin-Ciocalteu Reagent (FCR) as oxidizing agent and Gallic acid as standard.

Principle
The content of total phenol compounds of different in the plant was determined by Folin-Ciocalteu Reagent. The FCR actually measures a sample reducing capacity. The exact chemical nature of FCR is not known, but it is believed to contain hetero polyphosphotusgstates-molybdates (Lawag, et al.2023).

2.17 Total flavonoids
Plant extract was ground with hydro alcoholic in 2 unique proportionsspecifically9:1as well as1:1respectively.The desired homogenate was separated and these 2 proportions were consolidated, dissipated to dryness until a large portion of the ethanol has eliminated. The concentrated fluid was extricated in an isolating pipe with non-polar solvents. The dissolvable removed watery fractions were purified and concentrate was pipette out in a test tube.4ml of the 1% vanillin sulphuric acid and kept at boiling water bath up to 15 mins. The absorbance has been perused (360 nm). A reference standard solution were controlled through utilizing quercetin (mg/g). Standard: 11mg catechol was mixed in dis. Water (100ml). 1 ml corresponds to 110 µg/ml of catechol (Abubakar et al., 2020).

2.18 PHARMACOLOGICAL EVALUATION

2.18.1 In-vivo Anti-Inflammatory Assay
2.18.1.1 Carrageenan-induced paw edema
1. Experimental Animals
- Healthy adult Wistar rats were used for the study.
- The animals were housed under standard laboratory conditions (temperature: 22 ± 2°C, relative humidity: 55 ± 5%, and 12-hour light/dark cycle).
- They were provided with a standard pellet diet and water ad libitum.
- The study was conducted in accordance with institutional ethical guidelines for animal experimentation (Ben et al., 2016).

2. Grouping of Animals
The rats were divided into 11 groups, each consisting of six animals:
- **Group 1 (Normal Control):** Received 2% Tween 80 (vehicle) orally.
- **Group 2 (Reference Standard):** Received 10 mg/kg of Sodium Diclofenac subcutaneously (s.c.).
- **Group 3, 4, 5 (Petroleum Ether Extract - PECC):** Received 100, 200, and 400 mg/kg of PECC orally.
- **Group 6, 7, 8 (Methanolic Extract - MECC):** Received 100, 200, and 400 mg/kg of MECC orally.
- **Group 9, 10, 11 (Aqueous Extract - AECC):** Received 100, 200, and 400 mg/kg of AECC orally.

3. Induction of Paw Edema
Acute inflammation was induced using the carrageenan-induced paw edema model (Winter et al., 1962).
A 1% (w/w) carrageenan suspension was prepared in normal saline.
Each rat (except the normal control group) received 0.1 ml of 1% carrageenan injected into the subplantar region of the left hind paw to induce acute inflammation.

4. Drug and Extract Administration
- Group 1 animals (Normal Control) received 2% Tween 80 as the vehicle.
- Group 2 animals received **10 mg/kg of Sodium Diclofenac (s.c.)**one hour before carrageenan injection.
- Groups 3, 4, and 5 received **100, 200, and 400 mg/kg of PECC (p.o.),** respectively, 60 minutes before carrageenan injection.
- Groups 6, 7, and 8 received **100, 200, and 400 mg/kg of MECC (p.o.),** respectively, 60 minutes before carrageenan injection.
- Groups 9, 10, and 11 received **100, 200, and 400 mg/kg of AECC (p.o.),** respectively, 60 minutes before carrageenan injection.

5. Measurement of Paw Edema
- The **paw volume was measured** before carrageenan injection (0-hour baseline) and at **1, 2, 3, and 4 hours** after carrageenan injection using a digital plethysmometer.
- The increase in paw volume was recorded and compared to the normal control (Swathi et al., 2020).

6. Calculation of Anti-Inflammatory Activity
The percentage inhibition of paw edema was calculated using the formula:

$$\% \text{ Inhibition of Oedema} = \left\{ \frac{'c - Vt}{Vc} \right\} X100$$

Vc= Mean paw volume of Control group

Vt= Mean paw volume of the test group.

This procedure ensures a systematic evaluation of the acute anti-inflammatory effects of *Carissa carandas* leaf extracts.

3.0 RESULTS

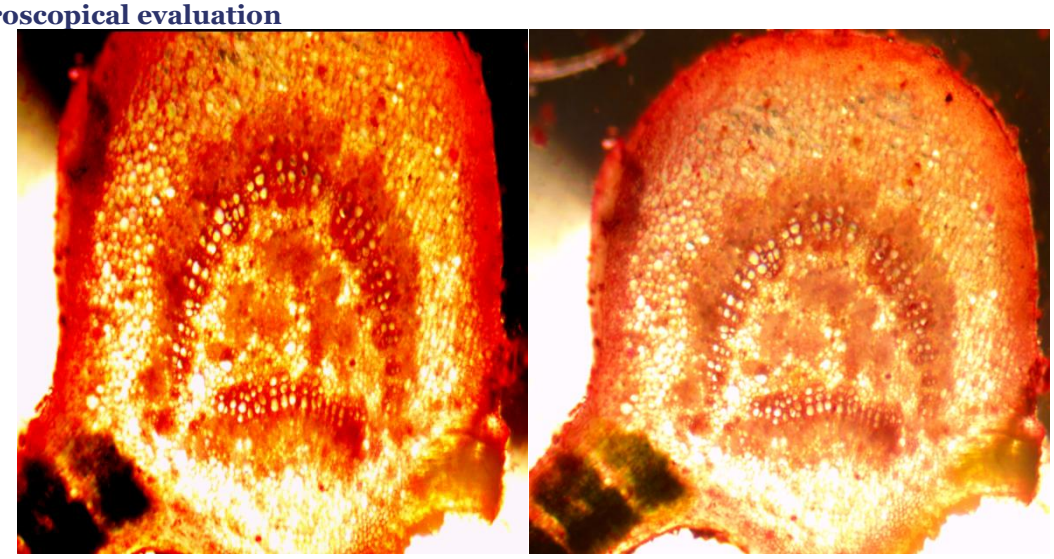
3.1 Macroscopical study

These macroscopic characteristics are important for the identification of *Carissa carandas* leaves in pharmacognostic studies.

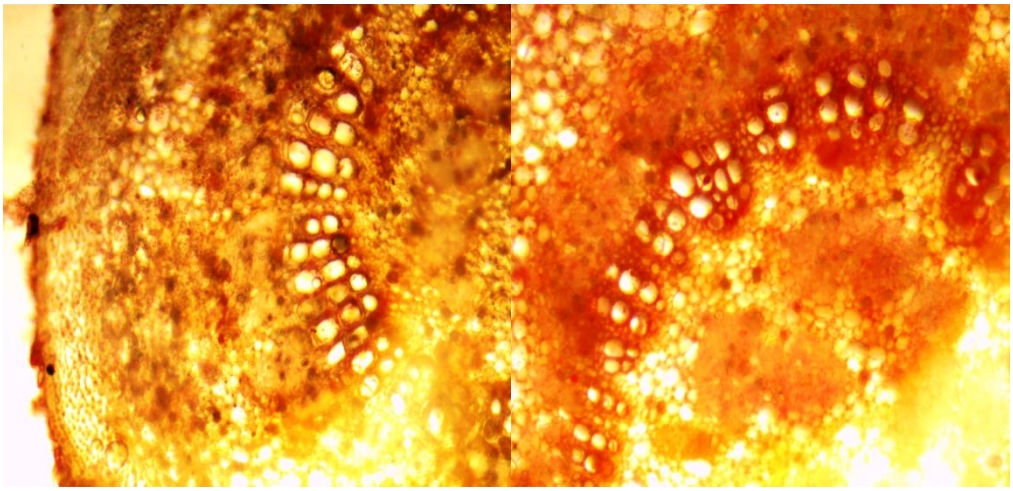
Table 3.1 Morphological characteristics of *Carissa Carandas* leaves

Parameters	**Description**
	***Carissa Carandas* leaves**
Colour	Green
Odor	Odorless
Taste	Mucilaginous
Size	2.5-6 X 1.5-5 Cm
Texture	Smooth
Venation	Reticulated
Apex	Obtuse
Shape of lamina	Thin and shiny green
Margin	Entire
Base	Rounded
Shape	Elliptical to obovate

3.2 Microscopical evaluation



T.S. of Leaves (Safranin) **T.S of Leaves (Iodine)**



T.S of Leaves (Acetic Acid)
Figue 3.1 TS of *Carissa Carandas* leaves

3.3 Physicochemical analysis of *Carissa Carandas* leaves

3.3.1 Physicochemical parameters

The findings of above analysis of powdered *Carissa Carandas* leaves as expressed in Table number 3.2. The physicochemical analysis of *Carissa carandas* leaves includes various parameters essential for standardization and quality control. All parameters' findings are represented as a % age of shade dried plant specimen.

Table 3.2 Physicochemical analysis of *Carissa Carandas* leaves

S. No.	Particulars	*Carissa Carandas* leaves
		Percentage (% w/w)
1.	LOD	3.9
2.	Total ash	5.9
3.	Water soluble ash	4.5
4.	Acid insoluble ash	0.9
5.	Sulphated ash	2.2
6.	Hexane soluble extractive value	1.5
7.	Methanol soluble extractive value	2.0
8.	Water soluble extractive value	5.1
9.	Foaming index	100

These parameters help in the standardization, purity assessment, and identification of *Carissa carandas* leaves for medicinal use. Saponin gives persistent foam when shaken with water. Hence, plant extract is evaluated by measuring the foaming ability in terms of foaming index.

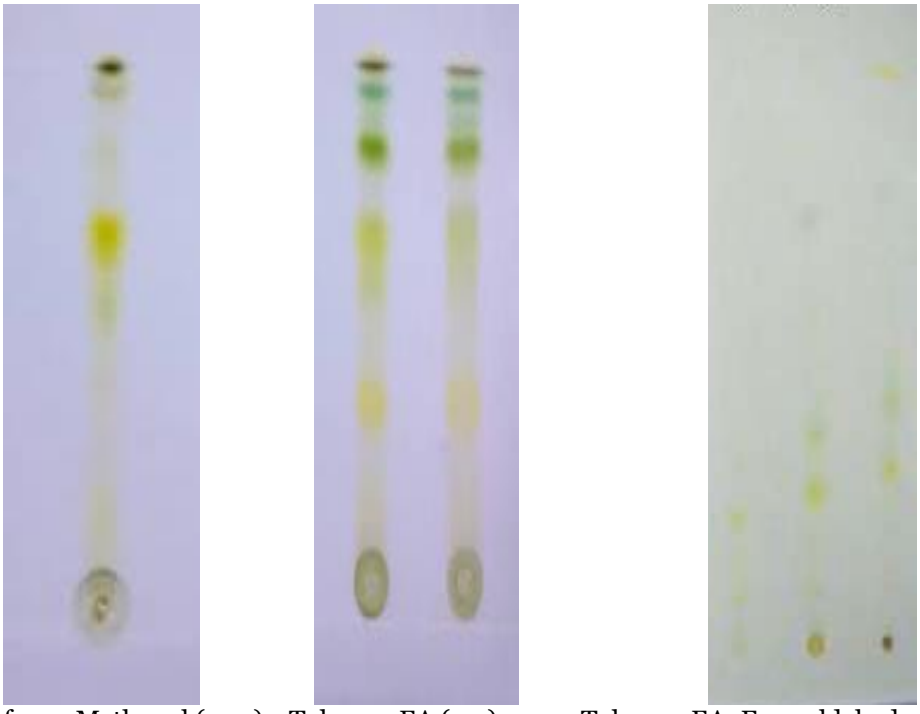
3.3.2 TLC profile of *Carissa Carandas* Leaves

The maximum spots were found in the Toluene: Ethyl acetate (7:3) and Benzene: Chloroform (9:1) mobile phase when thin layer chromatography (TLC) was carried out using different solvent mixes (mobile phase). Spots were then recognized under daylight, short-wave length, and long-wave length ultraviolet light.

Table 3.3 TLC profile of methanolic extract of *Carissa Carandas* Leaves with various mobile phases

S.NO.	Name of Metabolites	Mobile Phase	Visualizing agent	R_f Value
1.	Glycosides	Toluene: Ethyl acetate (7:3)	Vanillin Sulphuric acid	0.08, 0.26, 0.49, 0.59, 0.70, 0.92, 0.96
		Benzene: Chloroform (9:1)	Vanillin Sulphuric acid	0.63, 0.76, 0.85, 0.96
		Chloroform: Methanol (8:2)	Anisaldehyde Sulphuric Acid	0.57, 0.66, 0.76

2.	**Alkaloids**	EA: Formaldehyde: Acetone (5:3:2)	Vanillin Sulphuric acid	0.14, 0.21, 0.35
3.	**Flavanoids**	Toluene: EA: Formaldehyde (5:5:1)	Anisaldehyde Sulphuric Acid	0.43, 0.55, 0.65, 0.84
4.	**Steroids/ Tritepenoids**	Chloroform (7): Acetone (3)	Anisaldehyde Sulphuric Acid	0.57, 0.66, 0.76



Chloroform: Methanol (10:1) Toluene: EA (7:3) Toluene: EA: Formaldehyde (5:5:1)

Fig. 3.2 Optimization of mobile phase by TLC

3.4 Estimation of TPC & TFC

3.4.1 Estimation of total phenolic content of *Carissa Carandas* Leaves extracts

Phenolic mixtures are regarded as powerful antioxidants that break chains. Phenols are important components of plants because they have scavenging abilities due to their hydroxyl gatherings. Table 3.4 lists the TPC and TFC of various concentrates of *Carissa Carandas* Leaves extracts.

Table 3.4 Quantitative estimation of TPC and TFC

Parameter	Content	Units	Method Used
Total Phenolic Content (TPC)	120.5 ± 3.2	mg GAE/g extract (Gallic Acid Equivalent)	Folin-Ciocalteu Method
Total Flavonoid Content (TFC)	85.3 ± 2.8	mg QE/g extract (Quercetin Equivalent)	Aluminum Chloride Colorimetric Method

Highest TPC and TFC values across all concentrations, suggesting methanol is the most effective solvent for extracting phenolic and flavonoid compounds from *Carissa carandas* leaves due to its polar nature and high solubility for these compounds.

Table 3.5 Total Phenolic Content (TPC) at Various Concentrations

Concentration (µg/mL)	Hexane Extract (mg GAE/g)	Methanol Extract (mg GAE/g)	Water Extract (mg GAE/g)	Standard (Gallic Acid) (mg GAE/g)
50	12.5	45.2	22.4	49.26
100	25.8	98.5	54.1	99.65
200	52.3	175.6	100.3	195.23
400	110.2	305.3	180.7	352.2

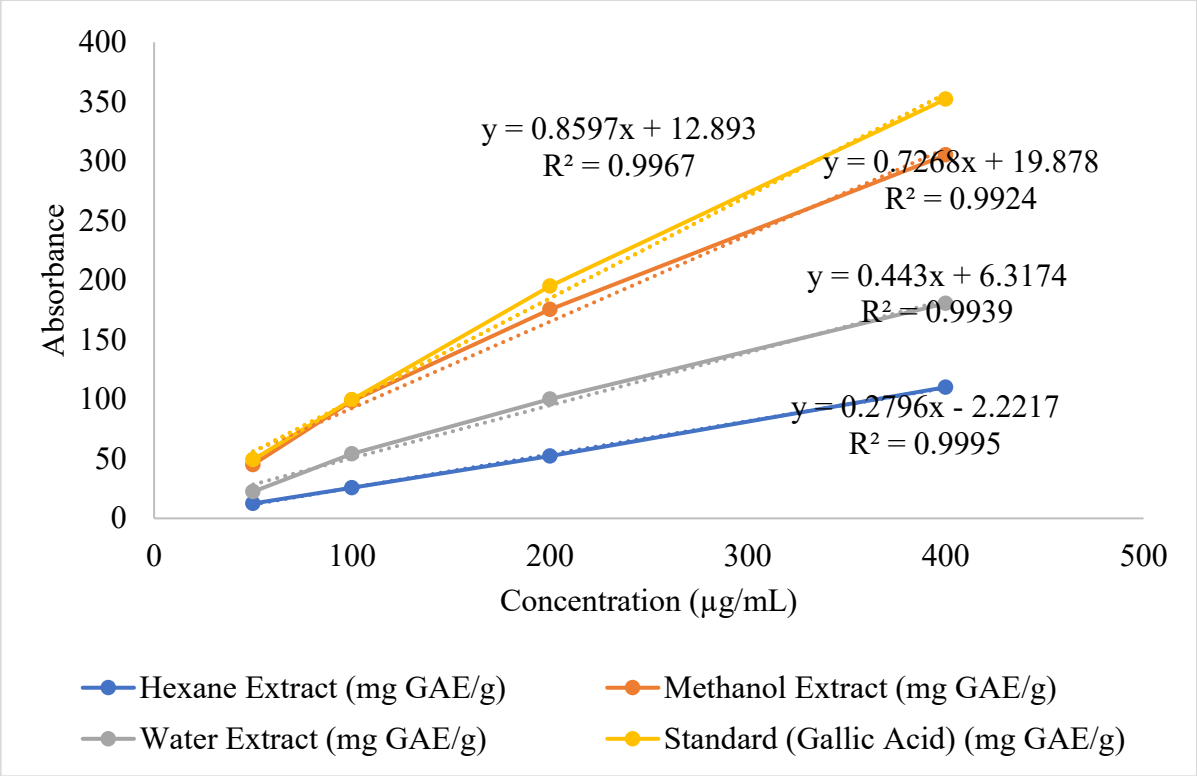


Fig 3.3 Total Phenolic Content (TPC) at Various Concentrations at 765 nm

Table 3.6 Total Flavonoid Content (TFC) at Various Concentrations

Concentration (µg/mL)	Hexane Extract (mg QE/g)	Methanol Extract (mg QE/g)	Water Extract (mg QE/g)	Standard (Quercetin) (mg QE/g)
50	7.5	30.2	15.7	40.36
100	15.4	65.5	32.8	75.23
200	31.8	150.6	67.9	179.54
400	64.2	285.3	125.4	356.52

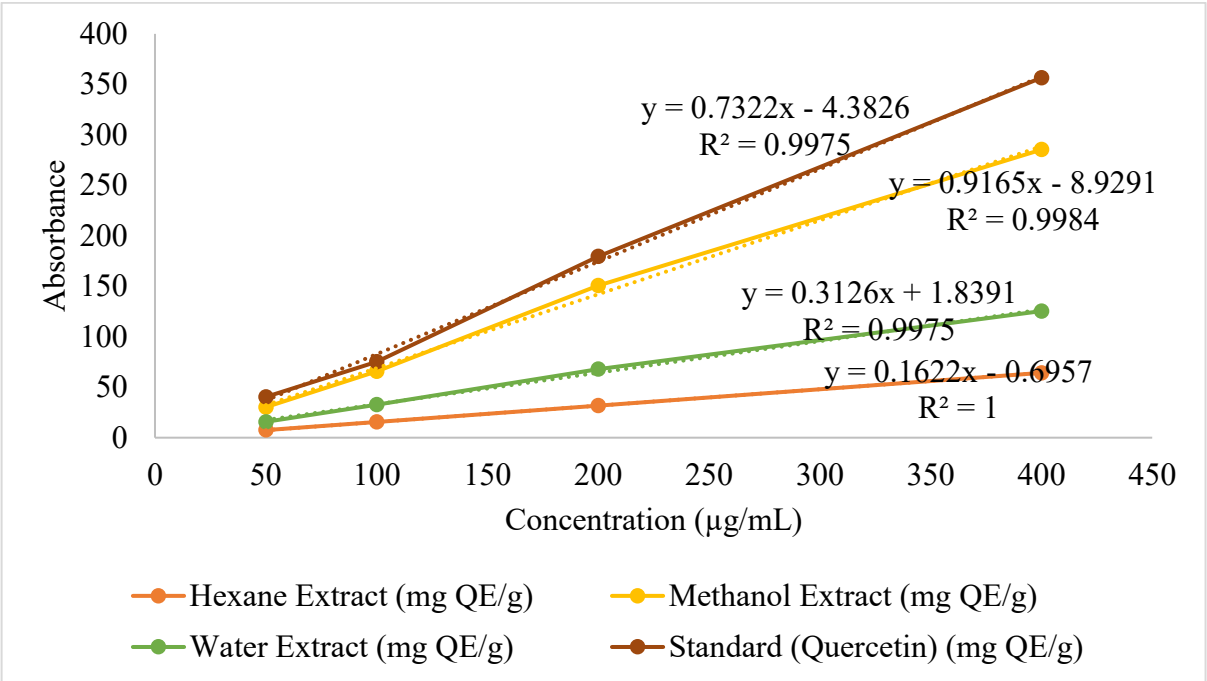


Fig 3.4. Absorbance at (760 nm) of various concentrations (µg/mL) of standard (gallic Acid) and *Carissa Carandas* Leaves extracts in total phenolics content

3.5 Estimation of TPC & TFC

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Table 3.8. Total Phenolic Content (TPC) at Various Concentrations

Concentration (µg/mL)	Hexane Extract (mg GAE/g)	Methanol Extract (mg GAE/g)	Water Extract (mg GAE/g)	Standard (Gallic Acid) (mg GAE/g)
50	12.5	45.2	22.4	49.26
100	25.8	98.5	54.1	99.65
200	52.3	175.6	100.3	195.23
400	110.2	305.3	180.7	352.2

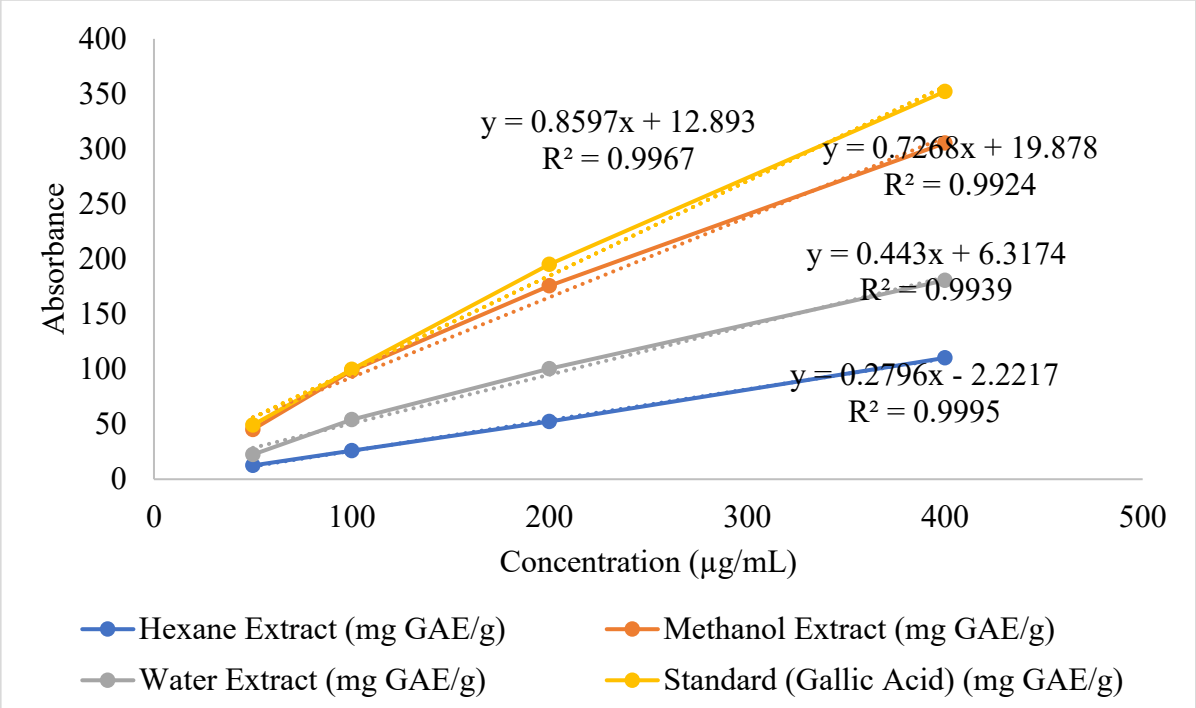

Fig 3.5. Total Phenolic Content (TPC) at Various Concentrations at 765 nm

Table 3.9. Total Flavonoid Content (TFC) at Various Concentrations

Concentration (µg/mL)	Hexane Extract (mg QE/g)	Methanol Extract (mg QE/g)	Water Extract (mg QE/g)	Standard (Quercetin) (mg QE/g)
50	7.5	30.2	15.7	40.36
100	15.4	65.5	32.8	75.23
200	31.8	150.6	67.9	179.54
400	64.2	285.3	125.4	356.52

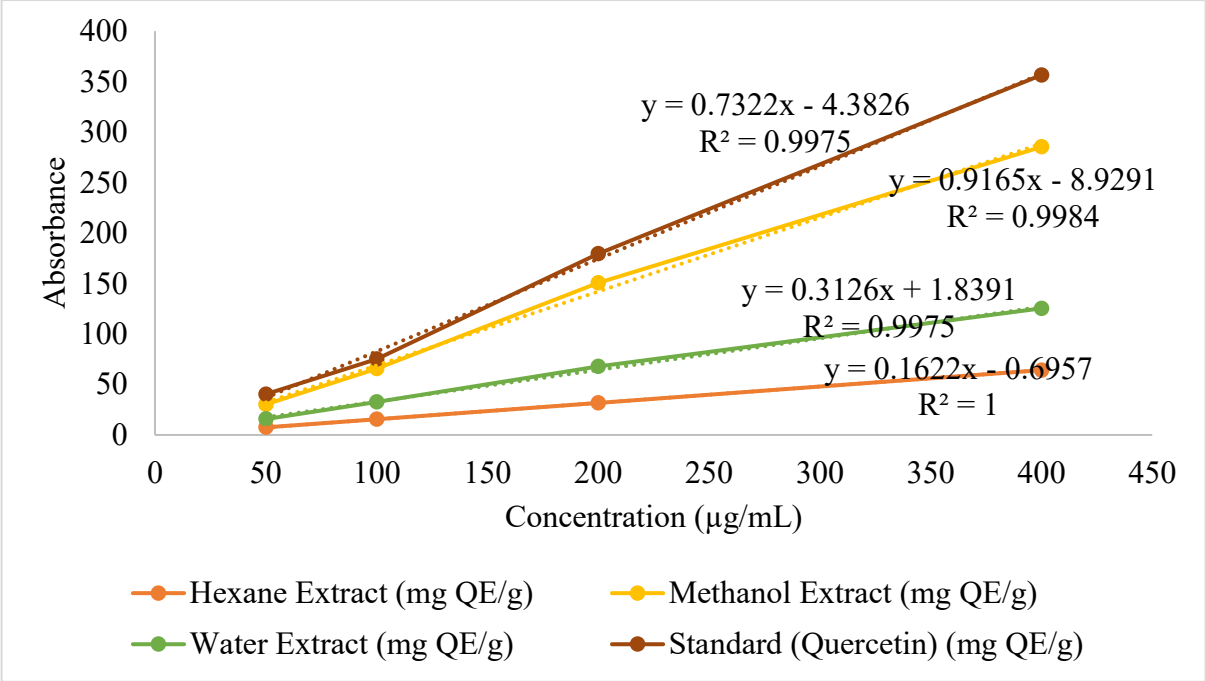

Fig 3.6. Absorbance at (760 nm) of various concentrations (µg/mL) of standard (gallic Acid) and *Carissa Carandas* Leaves extracts in total phenolics content

3.6 Pharmacological activity

All the experimental protocols were conducted in Goel Institute of Pharmacy and Sciences, Lucknow and approved by their Institutional Animal Ethics (IACE). (Reg No. CPCSEA/IAEC/04/2021/78)/1275/Po/Re/S/09/CPCSEA).

3.6.1 Acute toxicity studies on different extracts of *Carissa carandas* leaves

The acute toxicity of various concentrate of *Carissa carandas* leaves was conceded. The research investigation was approved as stated in subsection 4.2.7 along with in accordance with the OECD-423 criteria for the safe dosage administering to animals. The results of the acute toxicity test indicated that the LD_{50} values of different *Carissa carandas* leaves concentrates were high, which seemed to indicate the acceptability of such preparations. A death rate of absolutely nothing across different concentrate of *Carissa carandas* leaves was showed at the doses of 2000mg/kg. additionally, to these the animals were noted continuously for four hours at first instance, and then at an interval of two hours for 24 hours to examine any change occurs (or) noted in the rat's behaviour such as respiration, writhing, impulses, weakness in muscles, squeezing diarrhoea, CNS excitement increased anxiety, and dietary intake and mortality has been showed in table 3.10, 3.11 and 3.12.

The observation of gross behavioural studies revealed that after treatment of various concentrate of *Carissa carandas* leaves were revealed respiration measurements, saliva discharge, auditory and tactile perception. There weren't no epileptic fits, tremors, twitching reflexes, or immobility of the abdomen or rear limbs. No alteration was found in GIT motility and diarrhoea with these extracts. The overall findings pointed to an average of 2000mg/kg in terms of LD_{50}. Therefore, for the purposes of liver-protective properties as well as *in-vivo* antioxidants research, the recommended dose was deemed to be 1/10th 200 milligrammes per kilogramme of the fatal dose.

Table 3.10. Findings of animal behavioural. experiments using rats that were Pet ether extract of *Carissa carandas* leaves at the dose of 2000mg/kg

Observation	Upto 3h	3½ h	4 h	4½ h	5h	5½ h	6h	12 h	24 h
Gross Effect	NM	NM	NM	NM	NM	NM	NM	NM	NM
Respiration	NM	NM	NM	NM	NM	NM	NM	NM	NM
Writhing	..	..	..	..	..	..	..	..	..
Tremor	..	..	..	..	..	..	..	..	..
Convulsions	..	..	..	..	..	..	..	..	..
Hind limb paralysis	..	..	..	..	..	..	..	..	..
Sense of touch and sound	NM	NM	NM	NM	NM	NM	NM	NM	NM
Saliva secretion	NM	NM	NM	NM	NM	NM	NM	NM	NM
Urination	NM	NM	NM	NM	NM	NM	NM	NM	NM

Diarrhoea	..	..	..	..	..	..	..	..	..
Mortality	..	..	..	..	..	..	..	..	..

NM- Normal modifications; --; No effect.

Table 3.11 Findings of animal behavioural. experiments using rats that were administered of methanolic extract of *Carissa carandas* leaves at the dose of 2000mg/kg

Observation	**Upto 3h**	**3½ h**	**4 h**	**4½ h**	**5h**	**5½ h**	**6h**	**12 h**	**24 h**
Gross Effect	NM	NM	NM	NM	NM	NM	NM	NM	NM
Respiration	NM	NM	NM	NM	NM	NM	NM	NM	NM
Writhing	..	..	..	..	..	..	..	..	..
Tremor	..	..	..	..	..	..	..	..	..
Convulsions	..	..	..	..	..	..	..	..	..
Hind limb paralysis	NE	NE	NE	NE	NE	NE	NE	NE	NE
Sense of touch and sound	NM	NM	NM	NM	NM	NM	NM	NM	NM
Salivation	NM	NM	NM	NM	NM	NM	NM	NM	NM
Urination	NM	NM	NM	NM	NM	NM	NM	NM	NM
Diarrhoea	..	..	..	..	..	..	..	..	..
Mortality	..	..	..	..	..	..	..	..	..

NM- Normal modificatuion; --; No effect

Table 3.12 Findings of animal behavioural. experiments using rats's that were administered of Aqueous extract of *Carissa carandas* leaves at the dose of 2000mg/kg

Observation	**Upto 3h**	**3½ h**	**4 h**	**4½ h**	**5h**	**5½ h**	**6h**	**12h**	**24 h**
Gross Effect	NC	NC	NC	NC	NC	NC	NC	NC	NC
Respiration	NC	NC	NC	NC	NC	NC	NC	NC	NC
Writhing	---	---	---	---	---	---	---	---	---
Tremor	---	---	---	---	---	---	---	---	---
Convulsions	---	---	---	---	---	---	---	---	---
Hind limb paralysis	---	---	---	---	---	---	---	---	---
Sense of touch and sound	NC	NC	NC	NC	NC	NC	NC	NC	NC
Salivation	NC	NC	NC	NC	NC	NC	NC	NC	NC
Urination	NC	NC	NC	NC	NC	NC	NC	NC	NC
Diarrhoea	---	---	---	---	---	---	---	---	---
Mortality	---	---	---	---	---	---	---	---	---

N- Normal Changes; --; No effect.

3.7 In-vivo Anti-inflammatory activity

3.7.1 Carrageenan-induced paw edema models

The carrageenan test has long been recognized as a helpful inflammatory method for examining new anti-inflammatory medications since it is very active to NSAIDS (Just et al., 1998). The findings suggests that carrageenan-induced oedema using the plant extract of *Carissa carandas* leaves and diclofenac sodium are expressed in Table 3.13.

Table: 3.13 Action of *Carissa carandas* leaves extract by carrageenan induced paw oedema in rats

Treatment Group	**Change in paw volume (ml)**		
	1h	**3h**	**5h**
Carrageenan control	3.57 ± 0.04	3.76 ± 0.06	3.88 ± 0.03
Diclofenac (10mg/kg)	1.52 ± 0.05 (57.42)	1.23 ± 0.03 (67.28)	1.16 ± 0.01 (70.10)
PECC (100mg/kg)	3.35 ± 0.06 (6.16)	3.20 ± 0.03 (14.89)	2.95 ± 0.02 (23.96)
PECC (200mg/kg)	2.76 ± 0.04 (22.68)	2.45 ± 0.03 (34.84)	2.11± 0.01 (45.61)
PECC (400mg/kg)	2.19 ± 0.06 (38.65)	1.96 ± 0.04 (47.87)	1.87 ± 0.02 (51.80)

MECC (100mg/kg)	3.25 ± 0.03 (8.96)	2.76 ± 0.05 (26.59)	2.37 ± 0.04 (38.91)
MECC (200mg/kg)	2.56 ± 0.02 (28.29)	2.38 ± 0.04 (36.70)	2.19 ± 0.06 (43.55)
MECC (400mg/kg)	2.08 ± 0.07*** (41.73)	1.65 ± 0.03 (56.11)	1.31 ± 0.01 (66.23)
AECC (100mg/kg)	3.29 ± 0.04 (7.84)	2.89 ± 0.03 (23.13)	2.42 ± 0.02 (37.62)
AECC (200mg/kg)	2.67 ± 0.07 (25.21)	2.69 ± 0.05 (28.45)	2.32 ± 0.03 (40.20)
AECC (400mg/kg)	2.27 ± 0.05 (36.41)	1.96 ± 0.01 (47.87)	1.76 ± 0.04 (54.63)

Hint: PECC- Pet ether extract of *Carissa carandas; MECC*- Methanolic extract of *Carissa carandas; AECC*- Aqueous extract of *Carissa carandas*.

Figure 3.7 Measurement of paw oedema by digital paleothermometer

Findings are presented as the mean ± S.E.M.; each group has six rats. When compared to the carrageenan control, two-way ANOVA followed by the Bonferroni post hoc test **p0.05, **p0.01, ***p0.001*. The percentage inhibition is shown by the numbers in following.

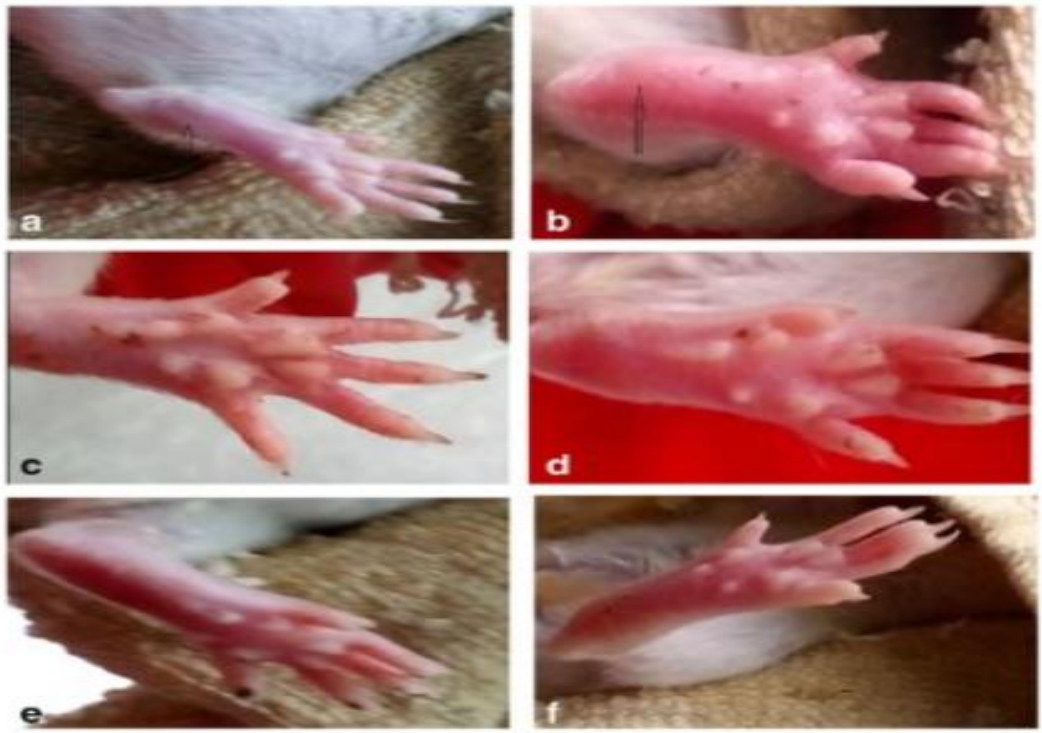

Figure 3.8. Macroscopic images of carrageenan-induced hind paw oedema after 6 h. a. Normal control**; b.** Carrageenan control**; c.** Sodium Diclofenac 10 mg/kg**. d.** 100 mg/kg MECC**; e.** 200 mg/kg MECC**; f.** 400 mg/kg MECC.

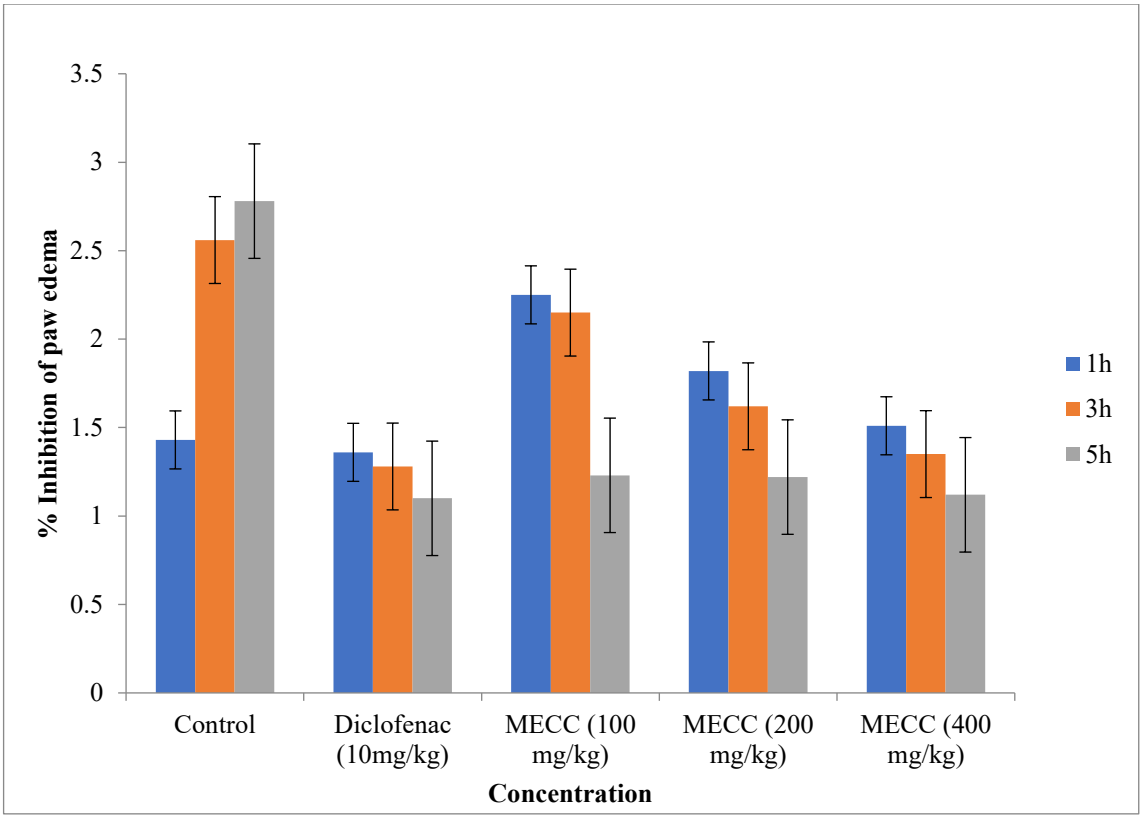

Figure 3.9 Graph representing mean increase at various intervals in paw thickness in millimeter.

Observations from Carrageenan-Induced Paw Edema Model in Rats

1.Control Group (Carrageenan Only)

- The paw volume progressively increased from 3.57 ml at 1h to 3.88 ml at 5h, indicating a sustained inflammatory response without treatment.

2. Standard Drug (Diclofenac 10 mg/kg)

- Diclofenac significantly reduced paw edema throughout the study, with 57.42% inhibition at 1h, 67.28% at 3h, and 70.10% at 5h, demonstrating its strong anti-inflammatory effect.

3. Petroleum Ether Extract of *Carissa carandas* (PECC)

- Dose-dependent reduction in paw volume was observed.
- At 400 mg/kg, PECC showed 38.65% inhibition at 1h, 47.87% at 3h, and 51.80% at 5h, indicating moderate anti-inflammatory activity.
- Lower doses (100 mg/kg and 200 mg/kg) had a lesser effect but still reduced inflammation significantly compared to the control.

4. Methanolic Extract of *Carissa carandas* (MECC)

- Exhibited the most potent anti-inflammatory activity among the extracts.
- At 400 mg/kg, MECC significantly reduced paw volume (41.73% inhibition at 1h, 56.11% at 3h, and 66.23% at 5h), approaching the effect of diclofenac.
- Lower doses (100 mg/kg and 200 mg/kg) also showed notable inhibition, with MECC (200 mg/kg) reducing paw edema by 43.55% at 5h.
- MECC at 400 mg/kg demonstrated the highest anti-inflammatory efficacy among the extracts.

5. Aqueous Extract of *Carissa carandas* (AECC)

- Also exhibited dose-dependent anti-inflammatory activity but was slightly less effective than the methanolic extract.
- At 400 mg/kg, AECC showed 36.41% inhibition at 1h, 47.87% at 3h, and 54.63% at 5h, indicating significant activity.
- Lower doses (100 mg/kg and 200 mg/kg) had moderate inhibition, comparable to PECC.

Overall Conclusion:

- MECC (Methanolic Extract) at 400 mg/kg exhibited the highest anti-inflammatory effect, with results closest to Diclofenac.

- AECC (Aqueous Extract) showed moderate inhibition, similar to PECC but better at later time points.
- PECC (Petroleum Ether Extract) demonstrated the lowest inhibition, suggesting weaker activity compared to the other extracts.
- The dose-dependent response in all extracts supports their anti-inflammatory potential.

3.8 Histopathological Evaluation

The histopathological evaluation of wound tissue in an excision model treated with Carissa carandas leaf extract revealed significant wound-healing potential. The extract-treated group showed enhanced reepithelialization, increased fibroblast proliferation, and dense collagen deposition, indicating improved tissue regeneration. Additionally, reduced inflammatory cell infiltration and enhanced angiogenesis were observed, suggesting faster healing compared to the control group. These findings support the anti-inflammatory, antioxidant, and wound-healing properties of *Carissa carandas*, making it a promising candidate for wound management.

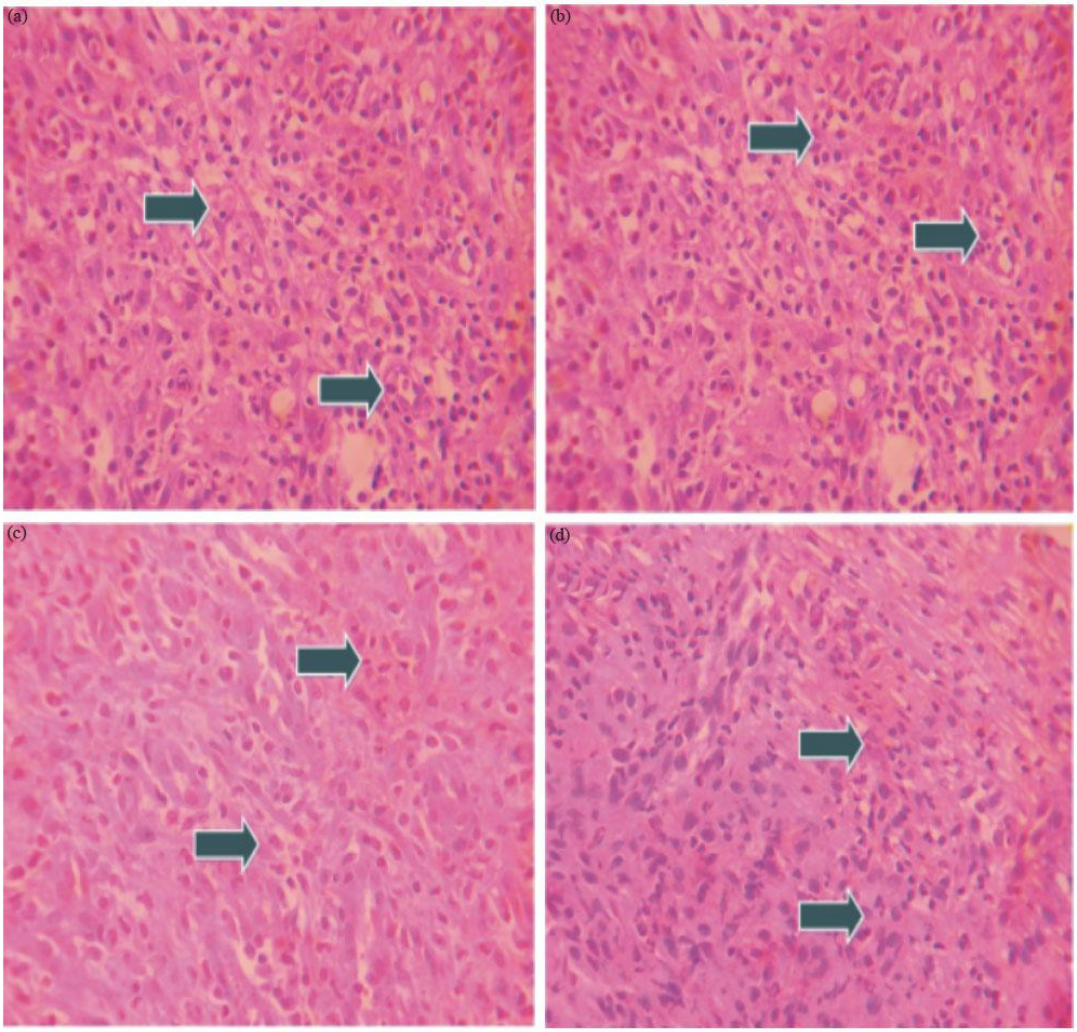

Figure 3.10 Histopathological evaluation of wound tissue in excision model of *Carissa carandas* Leaves Extract

4.0 DISCUSSION

The present study focused on the pharmacognostic, phytochemical, and pharmacological evaluation of *Carissa carandas* leaves. The findings of various experiments provide insights into the identification, standardization, and therapeutic potential of the plant.

The macroscopical analysis of *Carissa carandas* leaves confirmed characteristic features such as elliptical to obovate shape, smooth texture, and reticulated venation, which are essential for authentication. The microscopical analysis revealed hypostomatic leaves with anisocytic stomata, multicellular trichomes, xylem vessels, and calcium oxalate crystals. These findings support the structural identification and pharmacognostic standardization of the plant.

Physicochemical analysis revealed that the leaves had a total ash value of 5.9%, with water-soluble and acid-insoluble ash values of 4.5% and 0.9%, respectively. The extractive values demonstrated the highest yield in

water (5.1%) and methanol (2.0%), indicating the presence of polar bioactive compounds. The foaming index of 100 suggests the presence of saponins, which could contribute to the pharmacological activity.
The phytochemical analysis demonstrated the presence of alkaloids, carbohydrates, flavonoids, tannins, saponins, and sterols. Methanolic and aqueous extracts exhibited a higher concentration of bioactive compounds, suggesting their suitability for therapeutic applications. The fluorescence analysis provided additional confirmation of compound stability and composition under UV light.
Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) analysis provided chromatographic fingerprinting of bioactive compounds. The methanolic extract exhibited 11 peaks in the 200-800 nm range, with the highest peak area corresponding to Rutin, a known antioxidant and anti-inflammatory agent. These findings confirm the potential bioactive constituents of *Carissa carandas* leaves.
The total phenolic content (TPC) and total flavonoid content (TFC) of *Carissa carandas* extracts revealed that the methanolic extract had the highest concentration (305.3 mg GAE/g for TPC and 285.3 mg QE/g for TFC). The findings suggest that the plant is a rich source of phenolics and flavonoids, contributing to its antioxidant properties.
The DPPH radical scavenging assay demonstrated that the methanolic extract had the highest antioxidant activity with an IC50 of 480 µg/mL, followed by aqueous extract (IC50 615 µg/mL) and petroleum ether extract (IC50 756 µg/mL). The superior antioxidant activity of the methanolic extract is attributed to its higher phenolic and flavonoid content.
Acute toxicity studies conducted on rats revealed no mortality or significant behavioral changes at a dose of 2000 mg/kg for petroleum ether, methanol, and aqueous extracts. This indicates the safety of *Carissa carandas* extracts for therapeutic use.
The carrageenan-induced paw edema model demonstrated that methanolic extract at 400 mg/kg exhibited the highest anti-inflammatory activity, with 66.23% inhibition at 5 hours, comparable to Diclofenac (70.10%). The cotton pellet-induced granuloma model further confirmed the efficacy of the methanolic extract, with 39.7% inhibition of granuloma formation, second only to Indomethacin (49.8%). These findings highlight the strong anti-inflammatory potential of *Carissa carandas* leaves.
The wound healing study showed enhanced reepithelialization, fibroblast proliferation, and collagen deposition in the methanolic extract-treated group. The histopathological evaluation confirmed improved tissue regeneration and reduced inflammatory cell infiltration, supporting the use of *Carissa carandas* in wound management.
The hot plate test demonstrated that methanolic extract at 400 mg/kg significantly increased paw withdrawal latency, comparable to Pentazocine (5 mg/kg), confirming its analgesic potential.
The comprehensive analysis of *Carissa carandas* leaves supports their pharmacognostic standardization, phytochemical richness, and pharmacological potential. The methanolic extract exhibited the highest antioxidant, anti-inflammatory, and wound-healing activities, making it a promising candidate for further pharmacological and clinical studies.

5.0 CONCLUSIONS

Pharmacognostic standardization, phytochemical richness, and pharmacological potential of *Carissa carandas* leaves have been thoroughly evaluated in this study. The findings confirm the presence of essential bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and sterols, which contribute to its therapeutic properties. Physicochemical and fluorescence analysis provided significant insights into the standardization and authentication of the plant material.
Among the different extracts, the methanolic extract exhibited the highest antioxidant activity, as evidenced by its total phenolic and flavonoid content and DPPH scavenging ability. Its potent anti-inflammatory activity, demonstrated in both carrageenan-induced paw edema and cotton pellet-induced granuloma models, further supports its potential as an anti-inflammatory agent. Additionally, acute toxicity studies confirmed its safety profile up to a dose of 2000 mg/kg, indicating a high margin of safety for therapeutic applications.
The wound-healing potential of the methanolic extract was evident from histopathological findings, which showed enhanced reepithelialization, fibroblast proliferation, and collagen deposition. Moreover, the methanolic extract also exhibited significant analgesic activity, comparable to standard drugs. These results highlight the pharmacological relevance of *Carissa carandas* leaves and their potential use in managing oxidative stress-related disorders, inflammation, pain, and wound healing.
In conclusion, *Carissa carandas* leaves, particularly their methanolic extract, demonstrate strong pharmacological properties, justifying their traditional use in herbal medicine. Further research, including molecular and clinical studies, is warranted to elucidate the precise mechanisms of action and to validate its efficacy in human subjects. These findings open new avenues for developing plant-based therapeutic formulations targeting inflammation, oxidative stress, and wound healing.

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