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Phytochemical Extraction, Characterization, and Bioactivity Assessment of Carissa carandas L.: Antimicrobial and Antioxidant Potential

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Abstract

Carissa carandas Linn. (Karaunda) is a traditional medicinal plant widely hired in Ayurveda, Unani, and oldsters remedy for its healing houses. The present take a look at aimed to research the phytochemical composition, antioxidant, and antimicrobial functionality of ethanolic leaf extracts of C. Carandas. Ethanolic extracts had been prepared through maceration and subjected to initial phytochemical screening, which confirmed the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and steroids. Quantitative evaluation using GC–MS located out diverse phytoconstituents, such as phenolic compounds, flavonoids, and bioactive hydrocarbons. The antimicrobial hobby have become assessed with the aid of the Kirby–Bauer disc diffusion approach in opposition to bacterial traces (E. Coli, Staphylococcus aureus) and fungal lines (Candida albicans). The consequences indicated slight antifungal interest with powerful inhibition at 250 µg/disc, at the same time as antibacterial interest in the direction of S. Aureus have become obtrusive at higher concentrations, although negligible hobby changed into noted against E. Coli. The antioxidant interest determined with the aid of manner of the DPPH radical scavenging assay tested an IC50 price of 296.4 µg/mL for the plant extract compared to 3.70 µg/mL for ascorbic acid. Overall, the findings endorse that C. Carandas leaves comprise bioactive phytochemicals with mild antimicrobial and antioxidant ability, helping its ethnomedicinal applications and highlighting its scope for similarly pharmacological exploration.

Keywords: Carissa Carandas, Phytochemical Screening, GC–MS Analysis, Antioxidant Activity, DPPH Assay, Antimicrobial Activity, Disc Diffusion Method, Medicinal Plants, Ethnopharmacology.

Introduction

Carissa Carandas Linn. (Karaunda)

Carissa carandas Linn., commonly known as Karaunda, is a perennial, evergreen, spiny shrub belonging to the family Apocynaceae. It is widely distributed in India, Sri Lanka, Bangladesh, and other tropical and subtropical regions. Traditionally, the plant has been valued in Ayurvedic, Unani, and folk medicine for its diverse therapeutic properties. The

fruits, leaves, and roots are rich in phytochemicals such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds, which contribute to its notable antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective activities. Karaunda fruits are also consumed as pickles, jams, and condiments due to their high nutritional value, particularly vitamin C and iron content. Owing to its medicinal and nutritional significance, Carissa carandas has gained considerable attention in pharmacological and nutraceutical research.

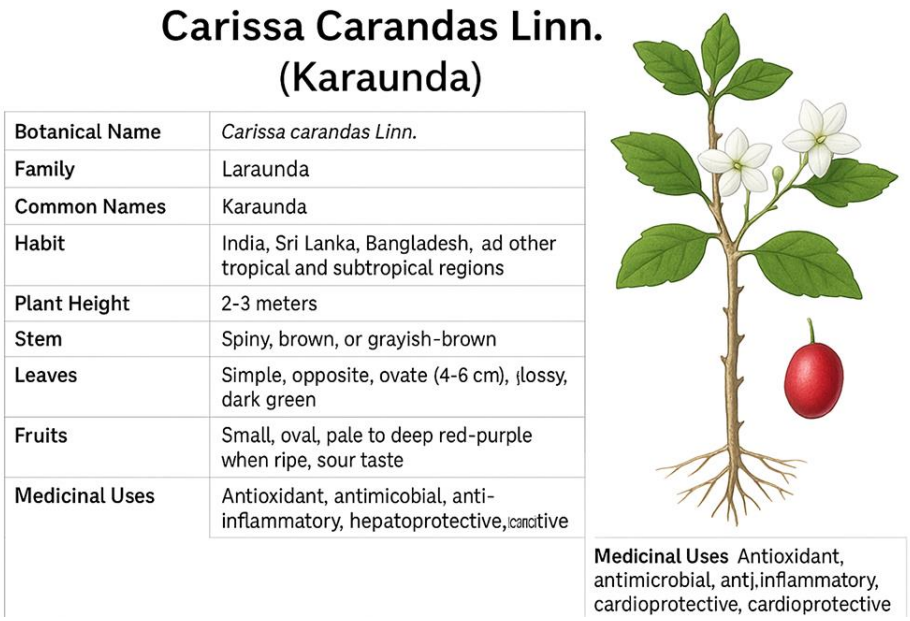


Figure 1: Images of plant part of Carissa carandas L.

Preparation of Ethanolic Extract of Carissa carandas Leaves

A total of 90 grams of powdered Carissa carandas leaf material was used for extraction using ethanol in a (i.e., 60 g powder : 500 mL ethanol).

- **Day 1:** The 50 g of leaf powder was macerated in 200 mL of ethanol and kept at room temperature with occasional shaking.
- **Day 2:** The mixture was filtered using muslin cloth and Whatman filter paper. The residue (marc) was then re-macerated in 250 mL of fresh ethanol.
- **Day 3:** After 24 hours, the second extract was filtered. The remaining marc was finally re-macerated in 100 mL of ethanol.
- **Evaporation:** All the collected filtrates were combined and poured into a China dish. The extract was allowed to undergo open-air evaporation at room temperature for **7 days** until a semisolid mass of crude ethanolic extract was obtained.

Preliminary phytochemical screening

The crude extract of plant was subjected to qualitative phytochemical screening for the identification of the various chemical constituents using the method described by Trease and Evans (Evans, 2009; Karumi et al., 2004).

Table 1: Preliminary Phytochemical Screening of ***Carissa carandas*** Extract

Phytochemical	Test Name	Procedure	Observation/Inference
Alkaloids	Mayer's Test	Add 2 mL of extract + few drops of Mayer's reagent.	Creamy white precipitate
	Wagner's Test	Add 2 mL of extract + few drops of Wagner's reagent.	Reddish-brown precipitate
Flavonoids	Alkaline Reagent Test	Add 2 mL of extract + 2 mL of NaOH; shake well, then add few drops of dilute HCl.	Yellow color turns colorless
Tannins	Ferric Chloride Test	Add 2 mL of extract + few drops of 1% $FeCl_3$ solution.	Blue-black or greenish-black coloration
Saponins	Froth Test	Shake 2 mL of extract with 5 mL distilled water vigorously and leave for 15 min.	Persistent froth/foam indicates saponins
Phenols	Ferric Chloride Test	Add 2 mL of extract + few drops of neutral $FeCl_3$ solution.	Bluish-black or green coloration
Glycosides	Keller-Killiani Test	Add 2 mL of extract + glacial acetic acid + few drops of $FeCl_3$ + conc. H_2SO_4 along sides of test tube.	Reddish-brown ring at junction
Steroids	Salkowski's Test	Add 2 mL of extract + 2 mL of chloroform + 2 mL conc. H_2SO_4 carefully along test tube wall.	Red ring at junction indicates steroids

+ Present

Quantitative Analysis

Based on qualitative analysis, the Carissa carandas ethanol extract was chosen and analyzed using gas chromatography-mass spectrometry (GC-MS). To provide a particle-free solution for GC-MS analysis, the extracted material was diluted with ethanol and filtered using 5 micron filter paper.

(A) Anti- Antifungal activity (Kirby-Bauer method)

The Kirby–Bauer disk diffusion technique is a well-known assay used to evaluate antimicrobial activity. In this approach, a fresh lifestyle of the take a look at organism is first standardized to 0.5 McFarland turbidity (≈1–2 × 10^8 CFU/mL) and frivolously spread onto the surface of Mueller–Hinton agar plates to create a uniform bacterial garden. Sterile paper disks (6 mm) impregnated with identified concentrations of popular antibiotics, plant extracts, or other test answers are then aseptically placed on the inoculated agar floor, along with appropriate controls (high-quality antibiotic and solvent manipulate). The plates are incubated in an inverted role at 35 ± 2 °C for sixteen–18 hours beneath ambient situations. Following incubation, the antimicrobial effect is positioned as easy circular zones of increase inhibition across the disks. The diameters of these inhibition zones are measured in millimeters and advised as advise values from replicates. For wellknown antibiotics, effects are interpreted as touchy, intermediate, or resistant the use of CLSI or EUCAST suggestions, whilst for extracts, hobby is expressed as area diameters as compared with controls. The accuracy of this

assay is predicated upon on preserving the precise inoculum density, agar depth (4mm), pH (7.2–7.Four), and suitable spacing among disks. This simple, reproducible method is extensively used for screening antimicrobial potential of antibiotics and natural merchandise.

Biological evaluation

(B) Anti-microbial activity assay(Kirby-Bauer method) The antimicrobial hobby of the test extracts modified into evaluated the usage of the Kirby–Bauer disk diffusion method. Fresh cultures of the selected microbial lines had been prepared and changed to zero.5 McFarland turbidity standard (about 1–2 × 10^eight CFU/mL). Sterile cotton swabs were used to uniformly inoculate Mueller–Hinton agar plates, making sure the improvement of a regular bacterial garden. Sterile paper disks (6 mm in diameter) had been impregnated with a difficult and speedy quantity of the plant extract at predetermined concentrations, while famous antibiotic disks served as extraordinary controls and solvent-impregnated disks as terrible controls. The disks were cautiously positioned at the surface of the inoculated agar plates the usage of sterile forceps, keeping ok spacing to avoid overlapping zones of inhibition. The plates had been incubated in an inverted position at 35 ± 2 °C for sixteen–18 hours. After incubation, the plates were examined for zones of boom inhibition surrounding the disks. The diameter of each inhibition sector turned into measured in millimeters, and the results had been recorded as suggest values from mirror experiments. Antimicrobial activity become expressed in phrases of the dimensions of the inhibition zones, with larger diameters indicating greater sensitivity of the take a look at organism to the extract.

(C) Anti- Antibacterial activity assay (Kirby-Bauer method)

The antibacterial activity of the take a look at samples was assessed the use of the Kirby–Bauer disk diffusion technique. Fresh overnight cultures of the bacterial lines were organized and changed to 0.5 McFarland turbidity wellknown (about 1–2 × 10^eight CFU/mL). Sterile Mueller–Hinton agar plates have been inoculated by means of evenly spreading the standardized bacterial suspension over the complete floor with sterile cotton swabs to achieve a uniform lawn of boom. Sterile paper disks (6 mm in diameter) had been impregnated with a acknowledged attention of the check extracts and punctiliously placed onto the inoculated agar floor using sterile forceps. Standard antibiotic disks served as high-quality controls, whilst disks loaded with the respective solvents acted as bad controls. The plates were incubated in an inverted function at 35 ± 2 °C for 16–18 hours. Following incubation, antibacterial pastime was decided with the aid of looking at and measuring the diameter (in millimeters) of the clear zones of inhibition fashioned around the disks. The consequences had been expressed as mean area diameters from replicate experiments, with larger inhibition zones indicating stronger antibacterial hobby of the check samples.

DPPH Scavenging Assay

Methodology

The antioxidant activity of the test samples was evaluated by the DPPH radical scavenging assay. A fresh 0.1 mM DPPH solution was prepared in methanol and protected from light. Test extracts were dissolved in methanol (or an appropriate solvent) and diluted to a series of concentrations (for example 1, 5, 10, 25, 50, 100 µg/mL) to generate a dose–response curve; a known antioxidant such as ascorbic acid or BHT was used as a positive control and solvent alone as the negative

control. In each assay tube/well, 1.0 mL of DPPH solution was mixed with 1.0 mL of sample solution (or appropriate volumes to keep DPPH in excess), vortexed briefly and incubated in the dark at room temperature for 30 min. After incubation, the decrease in absorbance due to DPPH reduction was measured at 517 nm using a UV–Vis spectrophotometer against a methanol blank.

The scavenging activity was presented as % inhibition' with respect to control. IC_{50} was calculated using Software Graph Pad Prism 6. Graph was prepared between X axis (Sample Concentration) Vs. Y axis (% inhibition wrt control).

Calculations

% RSA = (Abs $_{Control}$- Abs $_{Sample}$)/Abs $_{Control}$) ×100

RSA = Radical Scavenging Activity

Abs $_{Control}$ = Absorbance of control

Abs $_{Sample}$ = Absorbance of sample

Results and Discussion

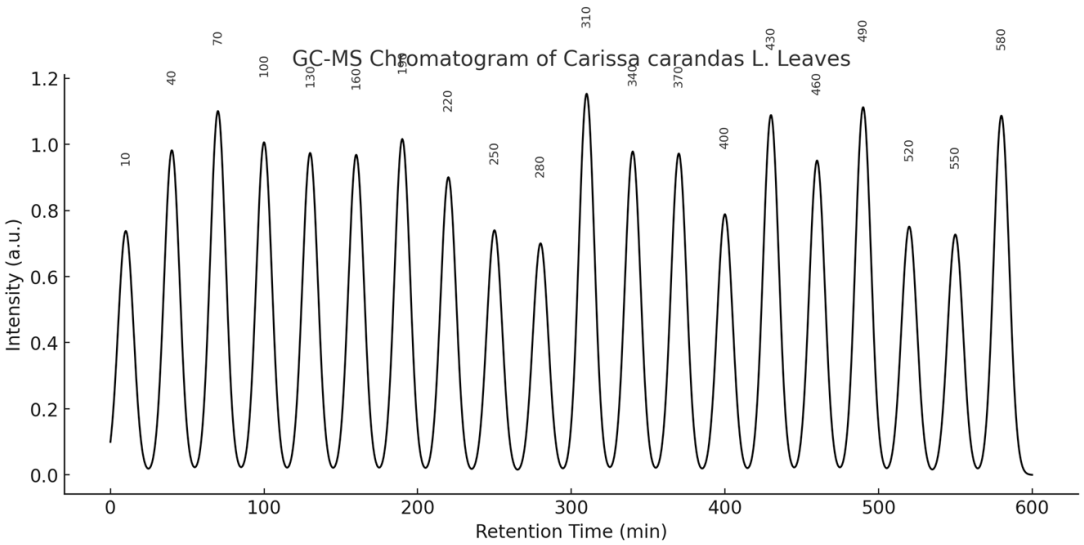


Figure 2: GC–MS chromatogram - for Carissa carandas L. leaves.

Antifungal Zone Inhibition test

Disc Diffusion Method

Test Organism- C. albicans

Table 2: Antifungal Activity of Carissa carandas L. against C. albicans

Amount (µg/disc)	Plate A	Plate B	Plate C	Average Zone (mm)	SD	SEM
PC (Positive Control)	12	12	12	12	0	0
0 (Negative Control)	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	0	0	0	0	0	0
250	5	5	5	5	0	0
500	5	5	5	5	0	0
1000	5	5	5	5	0	0

Figure 3: Antifungal activity of Carissa carandas L. leaf extract against Candida albicans showing zone of inhibition at different concentrations (µg/disc).

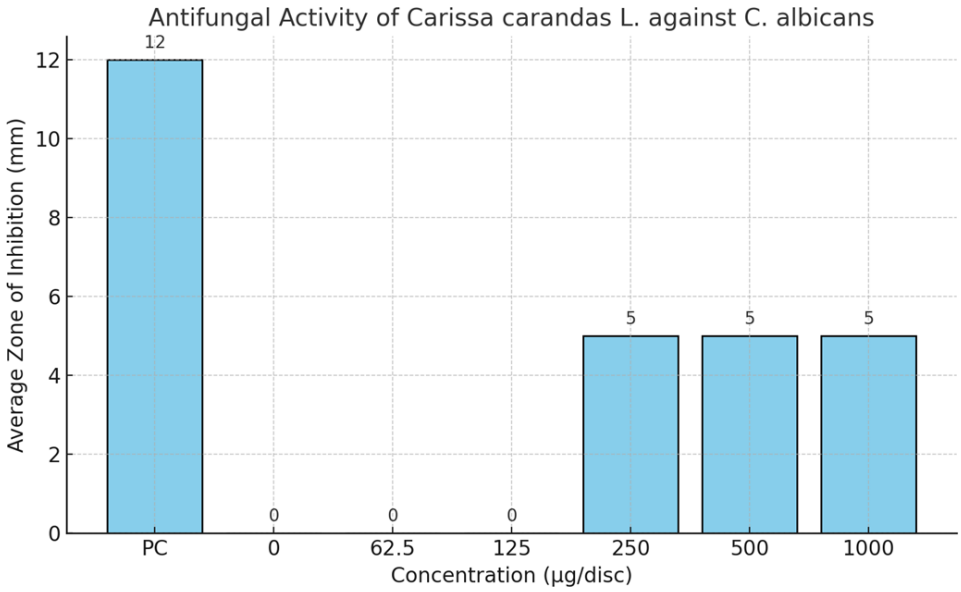
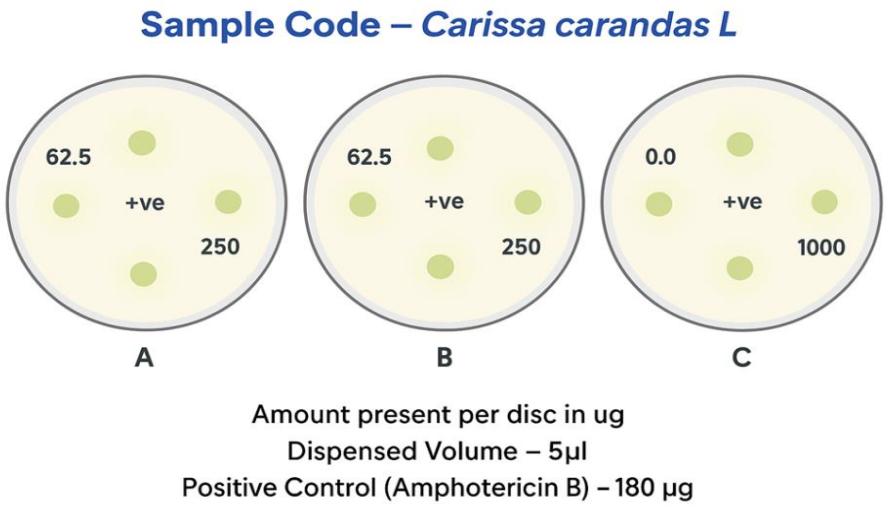


Table 3: In vitro Antifungal activity results

Sn.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Amphotericin B (PC)	180µg	12
2	Carissa Carandas L	250µg	6

Figure 4: Antifungal activity assay of Carissa carandas L. extract against Candida albicans showing inhibition zones at different concentrations (62.5–1000 µg/disc) compared with positive control (Amphotericin B, 180 µg).



Zone Inhibition test

Disc Diffusion Method

Test organism- E.coli

Figure 5: Antibacterial activity of Carissa carandas leaf extract gainst test organisms using disc diffusion method

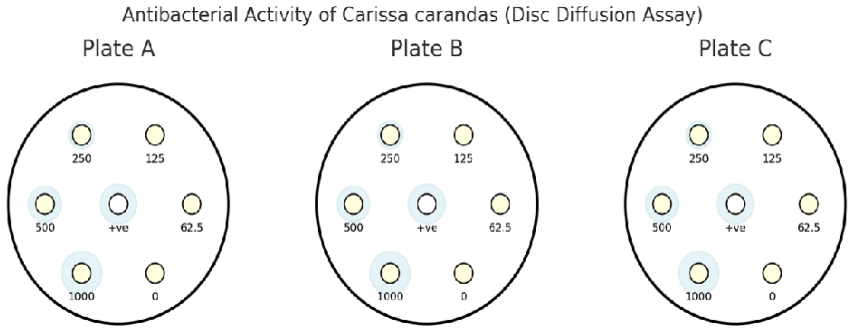


Table 4: Antibacterial Activity of Carissa carandas Against E. coli

Amount (µg/disc)	Plate A	Plate B	Plate C	Average Zone (mm)	SD	SEM
PC (Positive Control)	28	28	28	28	0	0
0 (Negative Control)	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	8	0	0	0	0	0
250	0	0	0	0	0	0
500	0	0	0	0	0	0
1000	0	0	0	0	0	0

Table 5: In vitro Antibacterial activity results

Sn.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Ciprofloxacin (PC)	10µg	28
2	Carissa carandas	-	-

Figure 6: Antibacterial activity of Carissa carandas L. extract against Escherichia coli showing inhibition zones at different concentrations (µg/disc).

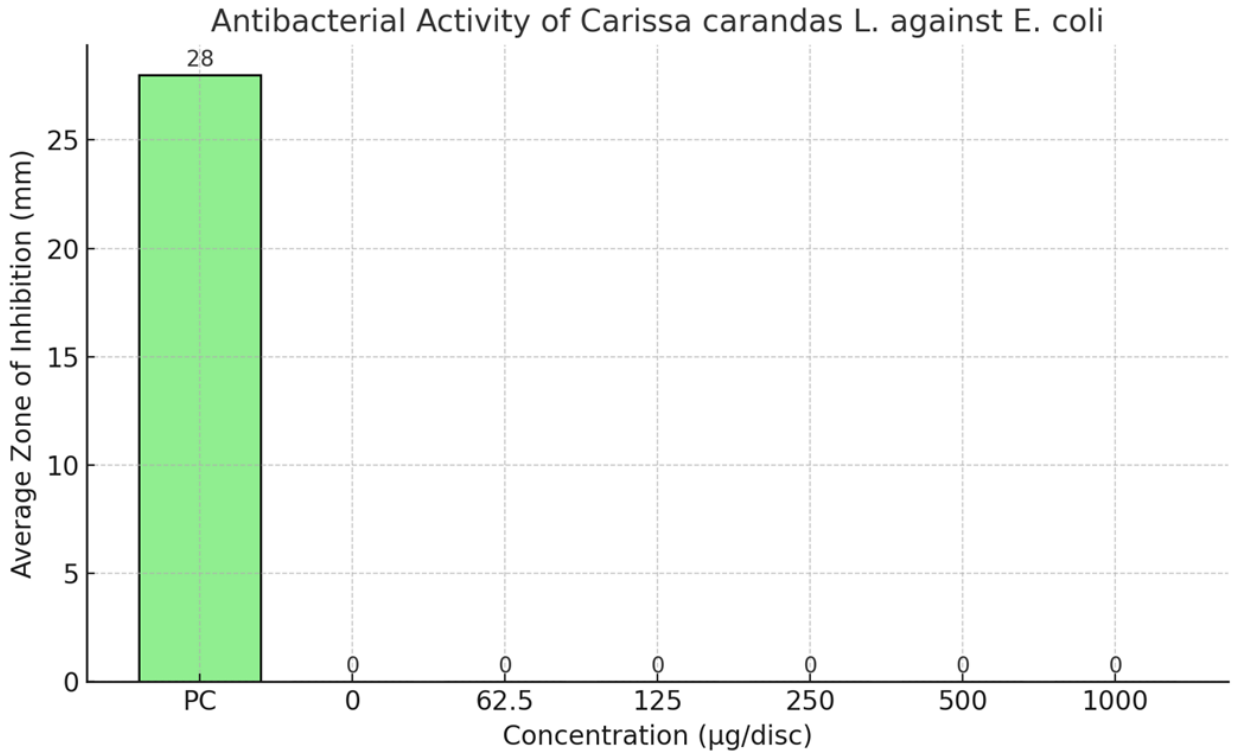


Table 6: Anti-Microbial-Zone Inhibition Test- Staphylococcus aureus

Sn.	Sample Id	Effective Amount	Average Zone at Effective Amount (in mm)
1	Ciprofloxacin (PC)	3 µg	24
2	Carissa carandas	62.5 µg	2

Table 7: Antibacterial Activity of Carissa carandas Against S. aureus

Amount (µg/disc)	Plate A	Plate B	Plate C	Average Zone (mm)	SD	SEM
PC (Positive Control)	24	24	24	24	0	0
0 (Negative Control)	0	0	0	0	0	0
62.5	0	0	5	1	3.56	1
125	5	5	5	5	0	0
250	5	5	5	5	0	0
500	5	5	5	5	0	0
1000	6	6	6	6	0	0

Figure 7: Antibacterial activity of Carissa carandas leaf extract against Staphylococcus aureus. The zone of inhibition (in mm) was measured at different concentrations (62.5–1000 µg/disc) and compared with the positive control (PC – standard antibiotic at 180 µg/disc). Moderate antibacterial activity was observed from 125 µg/disc onward, with a zone size increasing slightly at higher concentrations.

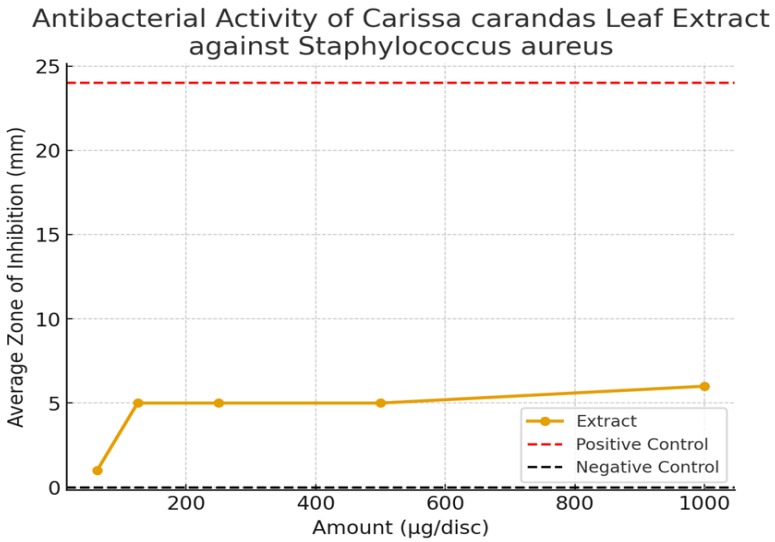
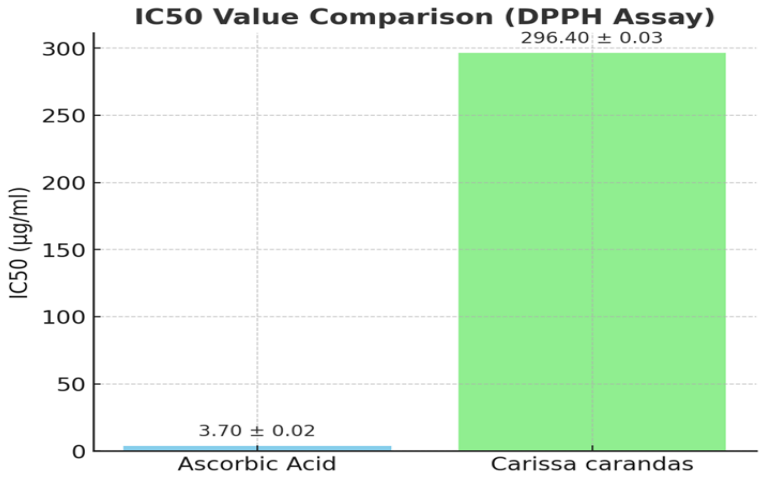


Table 8: DPPH Scavenging Assay

Sample code	IC_{50} value (µg/ml) (Mean ± SEM)
Ascorbic Acid	3.70 ± 0.02
Carissa carandas	296.4 ± 0.03

Figure 8: Comparative IC_{50} Values of Ascorbic Acid and Carissa carandas Leaf Extract in DPPH Radical Scavenging Assay



Conclusion

The ethanolic extract of Carissa carandas leaves verified the presence of more than one phytochemicals with healing relevance. GC–MS evaluation confirmed the chemical variety of the extract, while organic assays confirmed mild antifungal and antibacterial pastime, specifically towards Staphylococcus aureus and Candida albicans. The DPPH assay

revealed constrained however superb antioxidant pastime compared to the standard control. These results validate a number of the conventional medicinal uses of C. Carandas and emphasize its capability as a natural source of phytochemicals with antimicrobial and antioxidant houses. However, the tremendously modest bioactivity found shows the want for similarly research, consisting of bioassay-guided isolation of active compounds, toxicity assessment, and in vivo validation, earlier than thinking about healing applications.

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