

Chapter 3

Phytochemical screening and antimicrobial activity of *Carissa spinarum* L. (Apocynaceae): a wild therapeutic fruit

Sugimani Marndi^{1*}, Amardeep Kaur², Manish Kumar², Akshayjit Podder³, Debangshu Agrahari⁴, Brijesh Kumar⁵, Manvi Malwal⁶ and Remya Krishnan⁷

¹Ambika Prasad Research Foundation, Odisha, India

²S. D. College, Barnala, Punjab, India

³Rupai M.E. School, Under BEEO, Hapjan, Department of School Education, Assam, India

⁴Department of Dravyaguna, Jeevak Ayurved Medical College & Hospital Research Centre, Chandauli, Uttar Pradesh, India

⁵Social Forestry Division, Bulandshahr, Uttar Pradesh, India

⁶Post Graduate Government College for Girls 11 Chandigarh, India

⁷Daulat Ram College, Delhi University, Delhi, India

*Email-Id: sugimani2marndi@gmail.com

ABSTRACT

In a rapidly developing world of modern science, ethnobotany plays quite an important role. Preserving and conserving traditional botanical knowledge is as important as discovering new drugs. In fact, it enriches and complements these discoveries. *Carissa spinarum*, is one such understudied species of family Apocynaceae which has been long used in folk medicinal practices. The fruits of *C. spinarum* were analysed in this context for studying its phytochemical profile and antibacterial activity. Results showed that polar solvents have diverse range of bioactive compounds in comparison to the non-polar. The efficacy was validated by performing antibacterial assay against *Escherichia coli* using hydroethanolic extract. The minimum inhibitory concentration (MIC) was determined to be 25 mg/mL. Further analysis on its compound extraction and other pharmacological aspects is needed for concise evaluation and deeper insights.

Keywords: Ethnobotany, pharmacology, phytochemistry

INTRODUCTION

Ethnobotanical knowledge when transmitted across generations weaves a web of connection that comprises all the interdependent living beings including humans and plants. Traditionally, human beings used this knowledge to cure diseases and lead a sustainable lifestyle while tending to the nature for its gifts. With time, human greed grew and the urge to exploit increased. And, this over exploitation of natural resources hindered the rejuvenation capacity of the existing flora as well as the medicinal knowledge associated with it leading to the gradual decrease of traditional medicine practitioners (Nishteswar, 2014). Alongside the view to conserve and document the remaining flora, we focused on the wild edibles in this series. *Carissa spinarum* is such a member of family Apocynaceae that has long been hidden in the wilds and is understudied for its pharmacological values (Kumar, 2022). Different plant parts exhibit distinct therapeutic uses, reflecting the diverse range of phytoconstituents present in the species (Table 1). The extensive ethnomedicinal applications of *Carissa spinarum* highlight its importance as a multipurpose therapeutic plant in traditional medicine across Asia and Africa (Sharma *et al.*, 2023). Various parts of the plant like roots, leaves, bark, fruits, and even the whole plant are utilized to treat a wide range of ailments, including infectious, metabolic, and inflammatory disorders (Sharma *et al.*, 2023; Ansari and Patil, 2018). The plant is used in managing conditions such as fever, jaundice, cardiac diseases, diabetes, rheumatism, and gastrointestinal infections suggests its pharmacological potential as an antimicrobial, antioxidant, anti-inflammatory (Feyissa and Melaku, 2016), and cardioprotective agent. Moreover, its role in treating reproductive and vector-borne diseases, along with ethnoveterinary applications reflects that *C. spinarum* possesses a wide spectrum of therapeutic potential.

Table 1: Traditional medicinal uses of different parts of *Carissa spinarum*

Plant part used	Traditional medicinal uses
Whole Plant	Used to treat venereal, respiratory, and gastrointestinal infections, fever, jaundice, hepatitis, cardiac diseases, diabetes, malaria, pneumonia, chronic joint pain, asthma, rheumatism, stomachache, cough, chest complaints, sickle-cell anemia,

	hypertension, kidney complications, diarrhea, worms, eye cataracts, gastric ulcers, and cancer. Also used against microbial infections such as herpes, gonorrhea, syphilis, rabies, typhoid, jaundice, and polio. Acts as an antivenin for snake bites and used to treat infertility, asthenia, and premature ejaculation in males (Sharma et al., 2023; Ansari and Patil, 2018).
Leaves	Used as a mosquito repellent; applied in treating ulcers, rheumatism, asthma, and respiratory complaints (Ansari and Patil, 2018).
Roots	Used for treating fever, diarrhea, stomachache, worm infestation, jaundice, and as an antivenin for snake bites. Also employed to stop bleeding after delivery and to relieve muscle cramps (Sharma et al., 2023).
Bark	Used for treating gastrointestinal disorders, inflammation, and infectious diseases due to its bioactive compounds (Ansari and Patil, 2018).
Fruits	Used to treat gastric ulcers, diarrhea, and microbial infections; also recognized for their hepatoprotective, and cardioprotective properties (Sharma et al., 2023; Ansari and Patil, 2018).
Ethnoveterinary	Used to treat anaplasmosis and worm infestations in wounded animals (Sharma et al., 2023).

In recent decades, there has been renewed scientific interest in traditional medicine due to its holistic approach, cultural acceptance, and the discovery of bioactive compounds that serve as prototypes for modern drugs. In that particular context, fruits of *C. spinarum* were analysed in this study for their phytochemistry and antibacterial activity.

METHODOLOGY

Collection and identification of plant material: Mature fruits of *Carissa spinarum* were collected from the natural forest stands of the Chandaka Dampara Wildlife Sanctuary, Odisha, India. The plant species (Figure 1) was taxonomically identified by the authors. The collected specimens were assigned a voucher number and deposited in the departmental herbarium for future reference.

Preparation of plant material: The berries (Figure 2) were thoroughly washed under running tap water to remove adhering soil particles and other

surface contaminants. The cleaned fruits were air dried under shade at ambient temperature (25 ± 2 °C) for 7–10 days to minimize thermal degradation of bioactive constituents. Dried fruits were coarsely powdered using a mechanical grinder, sieved to obtain uniform particle size, and stored in sterile, airtight glass containers under dry conditions to prevent moisture absorption and microbial contamination until further analysis.



Figure 1: Fruits of *Carissa spinarum*



Figure 2: Collected fruits of *Carissa spinarum* for experimental use

Solvent extraction: The powdered fruit material was subjected to solvent extraction using the Soxhlet method (Jena et al. 2025). Two solvent systems of differing polarity were employed to ensure broad-spectrum extraction of phytoconstituents:

- a) n-Hexane
- b) Hydroethanol (7:3)

For each extraction, powdered material was placed in a thimble and was subjected to soxhlet extraction using the two above mentioned solvents. The extracted material was collected and was placed in room temperature for evaporation of extra solvent. The remaining extracts were stored in sterile glass vials under refrigerated conditions (4 °C) until further experimental use.

Qualitative phytochemical screening: Preliminary phytochemical screening of both n-hexane and hydroethanolic extracts was performed following standard protocols (Jena *et al.*, 2025) to detect the presence of major classes of secondary metabolites.

Determination of antibacterial activity: The antibacterial potential of the extracts was evaluated against *Escherichia coli* (MTCC 45) obtained from the Microbial Type Culture Collection, Chandigarh, India. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method as described by Jena *et al.*, (2025) with minor modifications.

RESULTS AND DISCUSSION

Depending on solvent polarity, the qualitative phytochemical screening of *Carissa spinarum* fruits was analyzed which detected the difference in secondary metabolite profiles (Table 2).

Table 2: Detection of secondary metabolites of *C. spinarum* fruit extract

Polarity	Solvent extract	Detected secondary metabolites	Phytochemical richness
Non-polar	n-Hexane	Reducing sugars	♦

Polar	Hydroethanolic	Tannins, Saponins, Phenolic compounds and Reducing sugars	◆
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(◆ = low, ◆ = high)

The non-polar n-hexane extract showed a limited phytochemical spectrum where, reducing sugars was the only detected constituent, indicating a lower phytochemical richness. In contrast, the hydroethanolic (7:3) extract demonstrated a broader range of metabolites, showing tannins, saponins, phenolic compounds, and reducing sugars. The higher metabolite diversity in the polar extract suggested that polar solvents are more effective in extracting bioactive constituents from *C. spinarum* fruits. The antibacterial potential of the hydroethanolic extract of *C. spinarum* fruits was then evaluated against *Escherichia coli* by determining the minimum inhibitory concentration (MIC) (Table 3).

Table 3: Estimation of Minimum Inhibitory Concentration (MIC)

Hydroethanolic Extract	Concentration (mg/mL)				MIC (mg/mL)
	100	50	25	12.5	
<i>Carissa spinarum</i> (fruits)	NG	NG	NG	G	25
Nutrient broth	NG	NG	NG	NG	-
Microbial broth	G	G	G	G	-
Antibiotics	NG	NG	NG	NG	-

(NG: No growth of bacteria; G: Growth)

The results showed that bacterial growth was completely inhibited (NG) at concentrations of 25 mg/mL and above, establishing MIC at 25 mg/mL (Figure 3). At lower concentrations (12.5 mg/mL), visible bacterial growth (G) was observed, confirming concentration dependent inhibitory activity. Control assays showed no bacterial growth (nutrient broth and antibiotic standards) validating the experimental conditions, whereas the microbial

broth control showed normal growth, confirming the viability of the bacterial inoculum.

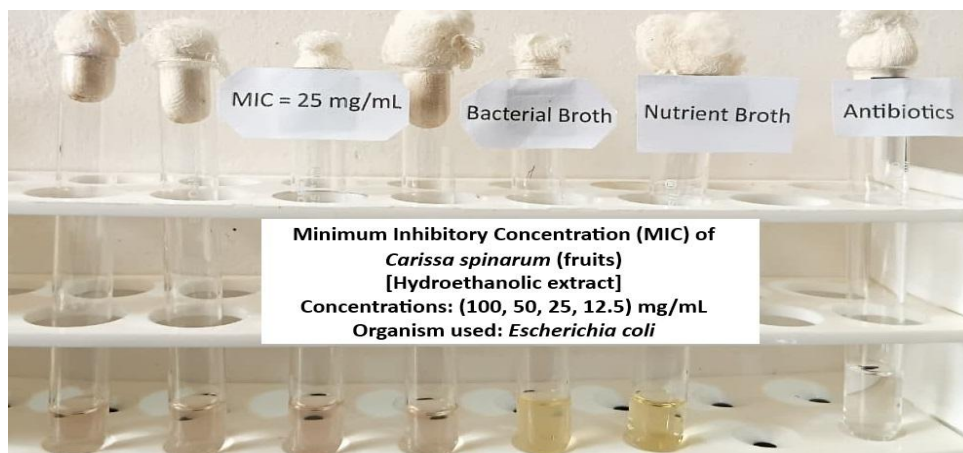


Figure 3: Minimum inhibition concentration (MIC) of fruits of *C. spinarum*

CONCLUSION

Rapid urbanization continues to exert pressure on wild habitats, while the rich knowledge of wild medicinal resources remains largely unexplored. In the present study, we profiled the phytoconstituents of *Carissa spinarum* fruits and assessed their phytochemical richness, which was further validated by determining the minimum inhibitory concentration (MIC) against *Escherichia coli*. Nevertheless, comprehensive scientific documentation, conservation of medicinal plants, and rigorous clinical evaluation are crucial to integrate traditional wisdom with modern medical science and to ensure the sustainable and responsible utilization of these bioresources in contemporary healthcare.

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