INFORMATICS JOURNALS

Short Review

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/55903

eISSN: 2320-3358 | Vol 26(10), 2026 | pp. 2673-2682

Bioactivity Profiling and Phytochemical Analysis of *Carissa carandas* Extracts: Antioxidant, Anti-inflammatory, and Anti-UTI Properties

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Abstract

Carissa carandas has long been esteemed in traditional medicine for its role in alleviating infections, inflammatory conditions, and disorders linked to oxidative damage, a benefit largely attributed to its varied and abundant phytochemical makeup. We conducted a thorough survey of published studies to characterise its chemical constituents, extraction approaches, and documented bioactivities, with an emphasis on antioxidant, anti-inflammatory, and Anti-Urinary Tract Infection (anti-UTI) actions. Reports indicate the plant is rich in biologically active classes including flavonoids, phenolic acids, alkaloids, tannins, and triterpenoids, which underpin its pharmacological effects. Among various extraction protocols, methanolic and ethanolic solvents yielded higher phytochemical recovery and stronger biological responses. Antioxidant activity was confirmed through several *in vitro* techniques such as DPPH, ABTS, FRAP, and ORAC assays, while anti-inflammatory potential was demonstrated by inhibition of protein denaturation and stabilisation of biological membranes. Moreover, extracts exhibited notable antimicrobial effects against common UTI pathogens, especially *Escherichia coli* and *Klebsiella pneumoniae*. Pharmacological assessments indicate an overall acceptable safety profile; nonetheless, robust clinical evidence in humans remains insufficient.

Major Findings: *Carissa carandas* appears to be a valuable natural source of antioxidant, anti-inflammatory, and antimicrobial agents, but further clinical studies, extract standardisation, and optimised formulation development are required to advance its use in therapeutics and nutraceuticals.

Keywords: Anti-inflammatory, Anti-UTI, Antimicrobial Activity, Antioxidant, *Carissa carandas*, Medicinal Plants, Phytochemicals

Abbreviation: ABTS-2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); COX-2- Cyclooxygenase-2; DPPH-2,2-Diphenyl-1-picrylhydrazyl; FRAP- Ferric Reducing Antioxidant Power; MBC- Minimum Bactericidal Concentration; MIC- Minimum Inhibitory Concentration; NF-κB- Nuclear Factor Kappa B; ORAC- Oxygen Radical Absorbance Capacity; ROS-Reactive Oxygen Species; UTI-Urinary Tract Infection.

(Article Chronicle: Article Received on: 20.04.2026; Revised on: 02.09.2026; Accepted on: 16.10.2026)

1. Introduction

Advances in food technology now extend beyond preservation toward creating foods that actively support immunity and general well-being. The health-promoting attributes of foods stem not only from macronutrients and micronutrients but also from bioactive phytochemicals[1]. Medicinal plants remain a cornerstone of traditional healthcare and continue to supply numerous therapeutic and nutraceutical agents; approximately 80% of the world's population still incorporates herbal remedies into primary healthcare[2]. Increasing scientific interest in plant-based alternatives has arisen partly because prolonged use

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of synthetic drugs can produce adverse effects and toxicity associated with long-term use of conventional pharmaceuticals[3].

Carissa carandas (*Apocynaceae),* popularly called karonda, is an evergreen, spiny shrub native to tropical and subtropical Asia, including India. It features prominently in Ayurvedic, Unani, and folk medicine, where fruits, leaves, and roots are used to treat fever, diarrhoea, epilepsy, digestive complaints, and dermatological conditions[4,5]. The fruits are also nutritionally valuable, being a source of vitamins and minerals, which supports their use as both food and medicine[6]. Ethnobotanical records document the traditional application of *C. carandas* for inflammation, infection, and gastrointestinal problems, underscoring its therapeutic breadth[7].

The plant's pharmacological relevance is attributed to a spectrum of phytochemicals phenolic acids, flavonoids, anthocyanins, alkaloids, tannins, and terpenoids known for antioxidant and anti-inflammatory actions through free radical neutralisation and modulation of inflammatory mediators. Oxidative stress is implicated in chronic ailments such as diabetes, cardiovascular disease, and infections. Phenolics from *C. carandas* have shown radical-scavenging activity comparable to synthetic antioxidants like Butylated Hydroxytoluene (BHT) and ascorbic acid[8]. Given the high global incidence of Urinary Tract Infections (UTIs) and rising antibiotic resistance, natural antimicrobials are being actively explored as safer alternatives for UTI prevention and management.

Together, these data support the potential of *C. carandas* as a source of natural antioxidants, anti-inflammatory agents, and antimicrobials, justifying traditional use and encouraging development into nutraceuticals, functional food ingredients, or phytopharmaceuticals. Future work addressing molecular mechanisms and *in vivo* validation will further consolidate its role in preventive and natural therapies.

2. Botanical Description

Carissa carandas is a compact, thorny shrub of the dogbane family (*Apocynaceae*), typically reaching 2-4 m in height. Its branches exude a milky latex and bear sharp spines. Leaves are elongated, green above and brownish beneath. The plant produces showy white flowers measuring about 3-5 cm across, usually flowering between January and February (Figure 1). Fruits form in small clusters as round to oval berries containing 3-5 flattened, dark brown to black seeds. Immature fruits have a pinkish-white hue, ripening to red or deep purple depending on cultivar (Figure 2). Fruits mature around May–June and are frequently harvested unripe for use as a vegetable; fully ripe fruit is eaten fresh or processed into various products[9].

3. Phytochemical Profile

3.1 Primary Metabolites

Primary metabolites in *C. carandas* are vital for plant growth, development, and metabolism and also add to its nutritional and therapeutic properties. Analyses show that various plant parts, particularly fruits, contain appreciable amounts of carbohydrates, proteins, lipids, organic acids, and vitamins, which are essential for physiological functions. Fruits are notably rich in carbohydrates and pectin, contributing to caloric content and applicability



Figure 1. *Carissa carandas* L. showing leaves, flowers, and developing fruits.



Figure 2. *Carissa carandas* L. showing clusters of immature and mature fruits.

in functional foods; proteins and amino acids support metabolic and structural roles. Organic acids such as malic, citric, and tartaric acids impart characteristic tartness and aid digestion. Nutritional assessments have also detected vitamin C and essential minerals, which bolster antioxidant defences and overall health benefits[10].

3.2 Secondary Metabolites

Phytochemical investigations have identified a broad spectrum of bioactive secondary metabolites in *C. carandas*, including flavonoids (quercetin, rutin), phenolic acids (gallic acid, chlorogenic acid), triterpenoids (lupeol, carissone), saponins, and tannins[11]. Roots reportedly contain compounds such as 2-acetyl phenol, lignin, carinol, sesquiterpenes (carissone, carindone), lupeol, β-sitosterol, 16β-hydroxybetulinic acid, α-amyrin and β-sitosterol glycoside, and the acridone alkaloid des-N-methylnoracronycine[12,13]. Fruit constituents include carisol (an α-amyrin epimer), linalool, β-caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol, and β-sitosterol[14]. Crude methanolic leaf extracts have tested positive for alkaloids, flavonoids, gums, reducing sugars, saponins, steroids, and tannins[15]. Flower essential oil components include myrcene, limonene, camphene, carene, dipentene, farnesol, nerolidol, α-terpineol, citronellal, β-ionone, linalool, and geranyl acetate[16].

Representative compounds such as quercetin, rutin, gallic acid, chlorogenic acid, lupeol, and carissone are presented in **Figure 3.** These phytochemicals contribute to the plant's reported antioxidant, antimicrobial, anti-inflammatory, and therapeutic potential.

4. Extraction Procedure

Powdered plant material (100 g) was subjected to sequential extraction with methanol, ethanol, and distilled water (500 mL each) for 72 h at ambient temperature with occasional agitation. The extracts were filtered and concentrated under reduced pressure at 40°C using a rotary evaporator. Dried extracts were labelled as CCM (methanol), CCE (ethanol), and CCA (aqueous) and kept at -20°C until further analysis[17].

5. Bioactivity Profiling

5.1 Antioxidant Activity

Antioxidant capacity was assessed using multiple complementary assays for reliability and reproducibility. The DPPH radical scavenging assay measured

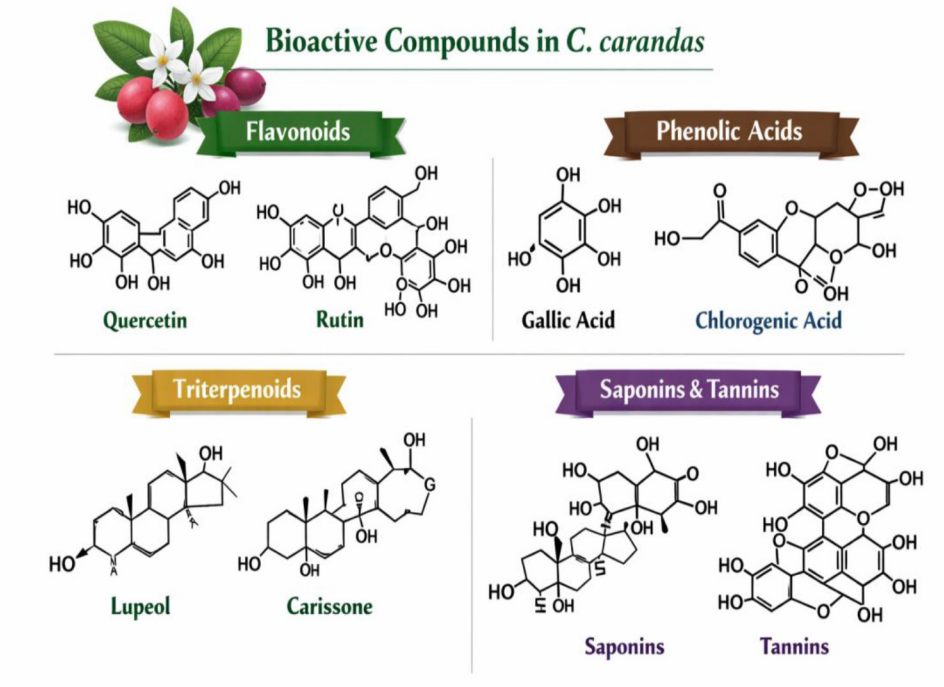


Figure 3. *Carissa carandas* contains diverse bioactive compounds, including flavonoids, phenolic acids, triterpenoids, saponins, and tannins.

absorbance at 517 nm and reported results as IC50 (µg/mL). The ABTS $^{+}$ radical cation assay was read at 734 nm with Trolox as the standard[18]. Ferric Reducing Antioxidant Power (FRAP) was measured at 593 nm[19], and the ORAC assay was performed using fluorescein decay kinetics and expressed in Trolox equivalents[20].

5.2 Anti-inflammatory Activity

Anti-inflammatory potential was explored using protein denaturation inhibition and membrane stabilisation assays. Protein denaturation was evaluated using Bovine Serum Albumin (BSA) with absorbance recorded at 660 nm. Human Red Blood Cell (HRBC) membrane stabilisation was quantified as percentage inhibition of haemolysis under hypotonic stress. Diclofenac sodium (100 µg/mL) served as a positive control[21].

5.3 Antibacterial and Anti-UTI Activity

Antimicrobial activity of *C. carandas* extracts was evaluated against common UTI pathogens including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*[22]. The agar well diffusion technique determined zones of inhibition, while MIC and MBC values were obtained via broth microdilution conforming to CLSI (2020) guidelines[23]. Ciprofloxacin (10 µg/mL) was used as the positive control .

6. Mechanism of Action

Table 1 shows that bioactive molecules in *C. carandas* operate through multiple and often overlapping pathways to produce antioxidant, anti-inflammatory,

Table 1. Mechanisms of action of bioactive compounds in *C. carandas*

Phytochemical class	Key compound	Mechanism of action	Biological effect	Molecular targets/ pathways	Outcomes	References
Phenolic acids	Gallic acid, chlorogenic acid	Scavenging of ROS and donation of hydrogen atoms/electrons	Antioxidant	Inhibition of lipid peroxidation; attenuation of oxidative stress	Reduction in ROS-mediated oxidative damage and enhancement of antioxidant protection	24,25
Flavonoids	Quercetin, rutin, myricetin	Inhibition of pro-inflammatory enzymes and modulation of inflammatory cytokines	Antioxidant; anti-inflammatory	COX-2 inhibition; NF-κB suppression; ↓ TNF-α and IL-6	Reduction of oxidative stress and inflammatory responses	26,27
Anthocyanins	Cyanidin and pelargonidin glycosides	Neutralisation of free radicals and stabilisation of cellular membranes	Antioxidant	ROS scavenging; modulation of endogenous antioxidant enzymes, including SOD and CAT	Reduction of oxidative damage and enhancement of antioxidant defence	28,29
Alkaloids	Indole-type alkaloids	Interference with microbial membrane integrity and essential microbial enzymes	Antimicrobial; potential anti-urinary tract infection activity	Alteration of membrane permeability; interference with microbial DNA replication and cellular enzymes	Suppression of microbial growth and reduction of microbial viability	30,31
Triterpenoids	Pentacyclic triterpenoids	Suppression of inflammatory mediators and modulation of apoptotic signalling	Anti-inflammatory; cytotoxic	Reduction of prostaglandin production; caspase activation and regulation of apoptotic pathways	Attenuation of inflammation and induction of apoptosis in susceptible cells	32,33

Table 1. Continued...

Phytochemical class	Key compound	Mechanism of action	Biological effect	Molecular targets/ pathways	Outcomes	References
Terpenoids and steroids	Phytosterols and terpenoids	Modulation of inflammatory responses and stabilisation of cellular membranes	Anti-inflammatory; antimicrobial	Cytokine regulation; membrane stabilization; modulation of inflammatory signalling	Reduction of inflammatory responses and maintenance of cellular membrane integrity	34
Other secondary metabolites	Tannins, saponins, glycosides	Protein precipitation, membrane interaction, and inhibition of microbial/ enzymatic activity	Antimicrobial; antioxidant	Cell-wall/ membrane disruption; enzyme inhibition; modulation of oxidative processes	Inhibition of microbial growth and protection against oxidative damage	35

and antimicrobial outcomes. Phenolic acids (*e.g.*, gallic and chlorogenic acids) exert antioxidant action by neutralising reactive oxygen species and preventing lipid peroxidation, thereby lowering oxidative burden. Flavonoids such as quercetin and rutin further attenuate inflammatory responses by inhibiting cyclooxygenase-2 (COX-2) and suppressing NF-κB signalling, which results in reduced secretion of pro-inflammatory cytokines like TNF-α and IL-6. Anthocyanins aid antioxidant defences by scavenging free radicals and upregulating endogenous enzymes such as superoxide dismutase and catalase. Alkaloids display antimicrobial properties relevant to UTIs by disrupting microbial membranes and interfering with nucleic acid replication. Triterpenoids contribute anti-inflammatory and cytotoxic effects through dampening prostaglandin production and engaging caspase-mediated apoptotic routes. Additionally, terpenoids, tannins, and saponins participate in immune modulation, membrane stabilisation, and microbial growth inhibition. Together, these multi-target actions underpin the therapeutic potential of *C. carandas* for managing oxidative stress, inflammation, and infectious diseases.

7. Ethnomedicinal Uses

Ayurvedic sources attribute *grahi* (absorbent) and *deepana* (digestive stimulant) properties to *C. carandas*, and it is traditionally employed for gastrointestinal and infectious disorders[36,37]. Fruits are used as antipyretics to treat fever and to address general weakness and dehydration. Unripe fruits, with their astringent nature, are commonly used for diarrhoea and dysentery, whereas ripe fruits have mild laxative and digestive benefits, enhancing appetite and gut function[38].

For urinary tract conditions, decoctions of roots and leaves have been administered for their antimicrobial and diuretic effects, facilitating pathogen clearance from the urinary tract[39]. Ethnomedicinal practice assigns specific roles to different plant parts: fruits for nutrition and digestive health; leaves for topical wound healing and internal use against infections; roots for analgesic, anti-inflammatory, and diuretic purposes; and bark for fever, skin ailments, and inflammatory disorders[40]. These varied traditional applications emphasise the plant's therapeutic versatility and provide rationale for continued pharmacological evaluation.

8. Experimental Evidence

A growing number of experimental studies support the pharmacological activities of *C. carandas in vitro* and *in vivo*. Several reports demonstrate notable antioxidant effects using assays such as DPPH, ABTS, and FRAP. For example, ethanolic fruit extracts displayed potent, dose-dependent free radical scavenging that correlated with high total phenolic and flavonoid levels[41]. Methanolic leaf extracts have been shown to reduce lipid peroxidation and elevate endogenous antioxidant enzymes like superoxide dismutase and catalase in experimental models[42].

Anti-inflammatory actions have been validated in animal studies; ethanolic root extracts produced

significant inhibition of carrageenan-induced paw oedema in rats, indicative of suppression of acute inflammatory reactions[43]. Additional investigations suggest such effects may involve downregulation of pro-inflammatory mediators including prostaglandins and cytokines. Leaf extracts have also been reported to lower nitric oxide production and other inflammatory markers in cell-based assays. Collectively, these findings demonstrate multi-target pharmacological activities for *C. carandas*, reinforcing its traditional uses and supporting its potential as a source of therapeutic bioactives.

9. Clinical Evidences

Clinical data for *C. carandas* are limited but provide preliminary indications of human benefits. A randomised dietary intervention examining consumption of *C. carandas* fruit products reported improvements in antioxidant status and reductions in oxidative stress markers among healthy volunteers, suggesting utility as a functional food to prevent oxidative stress related conditions[44]. Clinical evaluations of polyphenol-rich formulations containing *C. carandas* have shown favourable effects on inflammatory biomarkers, including decreases in serum C-Reactive Protein (CRP) and pro-inflammatory cytokines, implying potential anti-inflammatory activity[45].

Direct clinical trials for UTIs specifically involving *C. carandas* are scarce; however, herbal formulations including the plant have been associated with symptomatic relief and reduced UTI recurrence, likely reflecting combined antimicrobial and diuretic properties[46]. Traditional Ayurvedic preparations containing *C. carandas* have also been observed to improve gastrointestinal symptoms and reduce infection-related complaints[47].

Much of the clinical evidence remains indirect, derived from dietary studies, multi-herb products, or observational reports rather than rigorously controlled trials using standardised *C. carandas* extracts.

10. Safety, Toxicity and Side Effects

Toxicological evaluation of *C. carandas* has been conducted through various *in vivo* and *in vitro* studies to assess its safety profile. Acute, sub-acute, and sub-chronic toxicity studies using hydro alcoholic leaf extracts in animal models have demonstrated that the plant is generally safe at a wide range of doses. In experimental studies on Wistar rats, oral administration of *C. carandas* leaf extract at doses up to 1750 mg/kg and 5000 mg/kg did not produce any mortality or significant toxicological changes in vital organs such as the liver, kidney, heart, and spleen, as confirmed by histopathological analysis[48]. These findings suggest a high margin of safety for the plant extract in short-term and medium-term exposure.

In terms of safe dosage levels, pharmacological studies have shown that therapeutic effects of *C. carandas* extracts are typically observed at much lower doses ranging between 100-400 mg/kg in animal models, particularly in hepato-protective and antioxidant studies[49]. This finding suggests a substantial safety margin between effective and toxic doses, reinforcing its potential for therapeutic applications.

11. Discussion

The available literature indicates that *C. carandas* L. possesses a diverse phytochemical profile comprising phenolic acids, flavonoids, anthocyanins, alkaloids, triterpenoids, terpenoids, steroids, tannins, saponins, and glycosides. Phenolic and flavonoid constituents are associated with antioxidant activity through free-radical scavenging and modulation of oxidative processes. Anthocyanins may also contribute to antioxidant defence through ROS scavenging and enhancement of endogenous antioxidant mechanisms.

Triterpenoids and related phytoconstituents have been associated with anti-inflammatory activity through the modulation of inflammatory mediators and signalling pathways. Alkaloids, tannins, saponins, and other secondary metabolites may contribute to the reported antimicrobial activity of *C. carandas*, including potential activity against microorganisms associated with urinary tract infections. These biological activities are supported by phytochemical investigations and *in vitro* and *in vivo* experimental studies. Advanced analytical techniques, including LC-MS and GC-MS, have further contributed to the identification and characterization of bioactive constituents[50,51].

The available evidence further indicates that *C. carandas* possesses promising antioxidant,

anti-inflammatory, and antimicrobial activities, which may be associated with its phenolic, flavonoid, triterpenoid, alkaloid, tannin, and saponin constituents. Previous experimental studies have demonstrated antioxidant and antimicrobial effects of *C. carandas* extracts, supporting its traditional medicinal applications. However, variations in extraction methods, plant parts, concentrations, and experimental models may influence the reported activities and limit comparisons among studies. Advanced analytical techniques such as LC-MS and GC-MS have improved phytochemical characterisation and provided greater insight into the bioactive constituents responsible for the observed biological effects. Nevertheless, limited clinical evidence remains a major gap. Standardised extracts, mechanistic studies, toxicity evaluations, and clinical trials are required to establish the therapeutic potential of *C. carandas*[52].

12. Limitations

Despite promising pharmacology, the research on *C. carandas* has several constraints. A primary shortfall is the scarcity of well-designed clinical trials, with most evidence derived from *in vitro* assays and animal studies, limiting direct extrapolation to humans. There is considerable heterogeneity in extraction methods, varying solvents, techniques, and plant parts, resulting in inconsistent phytochemical profiles and divergent biological outcomes across studies. This variability complicates comparisons and reproducibility. Furthermore, many extracts lack standardisation for active constituents, dosage, and quality control metrics. Absence of standardised procedures and validated biomarkers impedes reliable formulation development and regulatory acceptance. Addressing these issues through methodical clinical trials, harmonized extraction strategies, and stringent standardisation is essential to fully validate the therapeutic utility and clinical applicability of *C. carandas*.

13. Future Perspective

Future investigations into *C. carandas* should emphasise clinical validation to translate encouraging preclinical results into evidence-based human therapies. Although numerous *in vitro* and *in vivo* studies have demonstrated antioxidant, anti-inflammatory, and antimicrobial effects, there is a clear need for robust, phased clinical trials to define pharmacokinetics, optimal dosing, long-term safety, and therapeutic efficacy, critical steps for drug development[53]. The plant has promising drug-discovery potential given its diverse phytochemical repertoire (flavonoids, phenolic, and triterpenoids). Recent research has identified bioactive compounds such as ursolic acid derivatives that display multi-target effects, including anticancer and anti-inflammatory actions via modulation of pathways like AKT1, HIF1A, and PTGS2, indicating *C. carandas* could supply lead compounds for novel phytopharmaceuticals and multi-target drugs[54,55]. Standardization of extracts is another priority to ensure batch-to-batch consistency and reproducible bioactivity; analytical techniques such as HPLC and LC-MS reveal that extraction conditions markedly affect concentrations of phenolics, flavonoids, and triterpenoids[56]. Therefore, establishing validated extraction protocols and selecting bioactive markers are necessary for quality control and regulatory approval. Emerging formulation technologies, including nanotechnology, provide avenues to enhance bioavailability and efficacy. For example, biosynthesized silver nanoparticles using *C. carandas* leaf extracts have exhibited superior antioxidant, antibacterial, and anticancer activities compared with crude extracts[57]. Nanocarriers (nanoparticles, liposomes, nano-emulsions) can improve stability, delivery, and target specificity of phytoconstituents. Incorporation of *C. carandas* into nutraceuticals and cosmeceuticals has also been explored, suggesting opportunities in the functional food and pharmaceutical sectors[58].

14. Conclusion

This review underscores *C. carandas* as a promising medicinal species with notable pharmacological potential supported by a rich phytochemical profile and diverse bioactivities. Bioactive constituents such as phenolic acids, flavonoids, alkaloids, and triterpenoids account for its antioxidant, anti-inflammatory, and antimicrobial actions, providing scientific validation for traditional uses in treating fever, digestive disturbances, and urinary infections. Experimental studies consistently demonstrate activity across multiple biological assays, and preliminary clinical observations

indicate potential health benefits in reducing oxidative stress and inflammation. Toxicology studies suggest a favourable safety margin. Nevertheless, the absence of rigorous clinical trials, variability in extraction methodologies, and limited extract standardization remain obstacles to clinical translation. Future work focused on clinical validation, identification of bioactive markers, and development of standardised formulations is imperative to ensure reproducibility and regulatory compliance. Advances in drug-delivery technologies, particularly nanotechnology-based systems, may further augment the bioavailability and therapeutic impact of *C. carandas* constituents. With coordinated efforts in clinical research, standardization, and formulation science, *C. carandas* could be developed into effective nutraceutical and phytopharmaceutical products for modern healthcare.

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Author Contribution

Dr. Shital Patel: Data curation, Conceptualisation, Formal analysis, Writing review and editing, writing-original draft, Visualisation, supervision, methodology. Dr. Anita Prakasam: Investigation, funding acquisition; Sumit Valand: Formal analysis, data curation, conceptualisation. Tanvi Panchori: Investigation, formal analysis; Sourabh Bhatt: Data curation. Krishna Patel: visualisation, validation, software; Rahul Patil: Supervision. All authors have read and agreed to the published version of the manuscript.