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ANALYSIS OF PHYTOCHEMICALS AND BIOPROSPECTING ACTIVITIES OF CARISSA CARANDAS L. FRUIT: QUALITATIVE PHYTOCHEMICAL PROFILING, HR-LCMS/MS ANNOTATION AND IN-SILICO DRUG-LIKENESS ASSESSMENT

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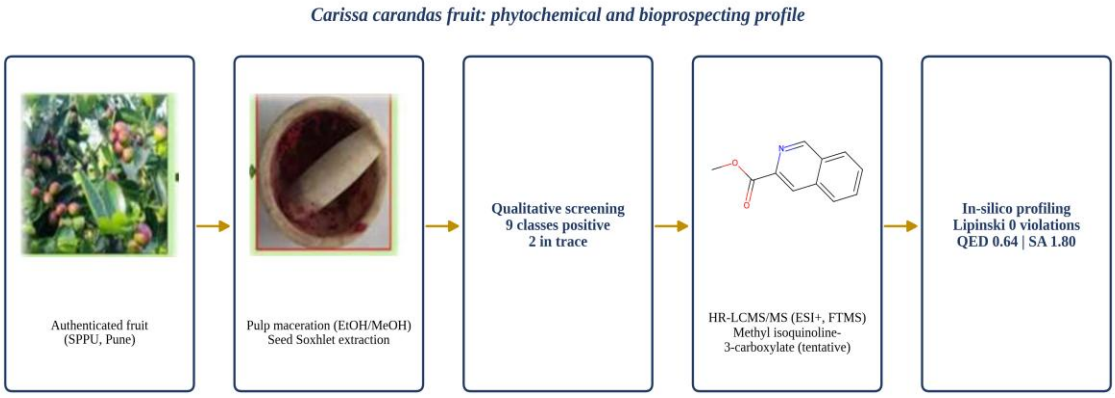
Highlights

- First combined qualitative, HR-LCMS/MS and in-silico profile of C. carandas fruit extract.
- Nine phytochemical classes detected; proteins and amino acids present in trace amounts.
- Methyl isoquinoline-3-carboxylate tentatively annotated (MSI level 3; mzCloud score 76.0).
- A dominant adduct family (M ≈ 414.194 Da) revealed at RT 12.24 min for future isolation.
- Annotated alkaloid shows zero Lipinski violations, QED 0.64 and no PAINS/Brenk alerts.

Graphical abstract



ABSTRACT

Carissa carandas L. (Apocynaceae; karonda) is an underutilised fruit shrub of the Indian subcontinent with a long record of use in Ayurvedic, Unani and folk medicine, yet instrumentally supported chemical profiles of its fruit remain limited. The present study was designed to document the phytochemical composition of authenticated C. carandas fruit and to generate an initial bioprospecting profile. Ripe fruits were authenticated, processed into pulp and seed fractions, and extracted by maceration (ethanol and methanol) and Soxhlet extraction, respectively. Qualitative screening revealed alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, steroids and carbohydrates, with proteins and amino acids in trace amounts. High-resolution LC-MS/MS (ESI^+, FTMS) with mzCloud spectral matching tentatively annotated methyl isoquinoline-3-carboxylate ($C_{11}H_9NO_2$; RT 5.076 min; mzCloud best match 76.0; mass error −23.18 ppm), assigned at Metabolomics Standards Initiative level 3. The full-scan spectrum at RT 12.24 min was dominated by a coherent adduct family ($[M+H]^+$, $[M+NH_4]^+$, $[M+K]^+$, $[2M+NH_4]^+$ and $[2M+K]^+$) whose back-calculated neutral mass converged on 414.194 ± 0.002 Da, identifying a major unannotated constituent for targeted isolation. In-silico profiling of the annotated alkaloid predicted favourable oral drug-likeness (MW 187.2 Da, WLOGP 2.02, TPSA 39.2 $Å^2$, zero Lipinski, Veber, Ghose and Egan violations, QED 0.64, synthetic accessibility 1.80) and no PAINS or Brenk structural alerts. Because methyl esters can arise from methanolic extraction, the natural occurrence of the annotated compound requires confirmation against an authentic standard and non-alcoholic extracts. Collectively, the data establish a reproducible extraction-to-annotation pipeline and prioritise C. carandas fruit constituents for quantitative and cell-based bioactivity evaluation.

KEYWORDS: Carissa carandas; karonda; phytochemical screening; HR-LCMS/MS; isoquinoline alkaloid; bioprospecting; drug-likeness; in-silico ADME

1. INTRODUCTION

Plant secondary metabolites remain one of the most productive sources of lead compounds in drug discovery; natural products and their derivatives account for a substantial proportion of small-molecule drugs approved over the last four decades [1]. Advances in high-resolution mass spectrometry, spectral libraries and computational screening have renewed interest in systematic bioprospecting of underexplored medicinal flora, allowing complex extracts to be profiled and prioritised far earlier and more cheaply than classical isolation-first approaches [2].

The genus Carissa (Apocynaceae) comprises evergreen shrubs and small trees distributed across tropical and subtropical Africa, Asia and Oceania, and phytochemical studies on nine species have yielded more than one hundred compounds, including triterpenes, cardiac glycosides, sesquiterpenes, lignans, sterols, flavonoids and simple phenolics [3]. Carissa carandas L., known as karonda (Hindi) or karvanda (Marathi), is a thorny shrub whose small berries turn from pink to purple-black on ripening and are consumed fresh or processed into pickles, jams and beverages [4,5]. Traditional systems attribute antipyretic, antiscorbutic, anthelmintic, hepatoprotective and digestive properties to the fruit, leaves and roots [5,6].

Pharmacological investigations have substantiated several of these uses: unripe fruit extract has shown antidiabetic potential [7], crude extracts exhibit spasmolytic and laxative activities that rationalise the traditional use in constipation and diarrhoea [8], methanolic leaf extract displays antioxidant and DNA-protective activity [9], and the isohopane triterpene carandinol isolated from the leaves was cytotoxic against cancer cell lines [10]. More recently, bioassay-guided fractionation coupled with LC-ESI-MS/MS tentatively identified phenolic acids, quercetin and anthocyanins as contributors to the antioxidant, anti-inflammatory and anti-urinary-tract-infection activities of C. carandas [11], and LC-MS/MS and GC-MS profiling of fruit varieties has been reported [12,13].

Despite this body of work, most studies have relied on crude-extract bioassays or leaf and root material, and comparatively few have combined authenticated fruit material, orthogonal qualitative screening, accurate-mass LC-MS/MS annotation with an explicit confidence level, and computational drug-likeness assessment of the annotated constituents within a single workflow. Such an integrated approach is important because it links a chemically defined extract to testable hypotheses about bioactivity and permits rational prioritisation of constituents for isolation and wet-laboratory assays.

The present study therefore aimed to (i) collect, authenticate and process C. carandas fruit into pulp and seed extracts; (ii) profile the major phytochemical classes by standard qualitative tests; (iii) annotate constituents by HR-LCMS/MS with transparent reporting of mass accuracy and identification confidence; and (iv) predict the physicochemical, pharmacokinetic and drug-likeness properties of the annotated compound as a basis for subsequent bioactivity evaluation.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Analytical-grade ethanol and methanol were used for extraction. Reagents for qualitative phytochemical tests (Mayer's and Wagner's reagents, ferric chloride, lead acetate, glacial acetic acid, concentrated sulphuric acid, chloroform, magnesium turnings, concentrated hydrochloric acid, Molisch's reagent, biuret reagent and ninhydrin) were of analytical grade and prepared according to standard pharmacognostic procedures [14,15]. LC-MS-grade solvents were used for HR-LCMS analysis.

2.2 Plant material and authentication

Fresh, ripe fruits of Carissa carandas L. were collected from the local region of Solapur, Maharashtra, India. The plant material was authenticated by Dr. Milind Sardesai, Department of Botany, Savitribai Phule Pune University (SPPU), Pune, and a voucher specimen was deposited for future reference.

2.3 Sample processing and extraction

Fruits were washed under running tap water to remove adhering debris and rinsed with distilled water to reduce surface microbial load. Pulp and seeds were separated manually (Fig. 2). The pulp was ground to a fine homogenate in a mortar and pestle to maximise the solvent-accessible surface area and divided into two portions that were macerated separately in ethanol and in methanol to yield parallel ethanolic and methanolic fruit extracts. The use of two protic solvents of differing polarity was intended to broaden the recovery of moderately polar (flavonoids, phenolics) and highly polar (glycosidic) constituents. Seeds were dried, partially crushed and reduced to a coarse powder, which was exhaustively extracted by continuous hot percolation in a Soxhlet apparatus. Extracts were filtered, concentrated and stored in airtight amber containers at 4 °C until analysis. The overall experimental workflow is summarised in Fig. 1.

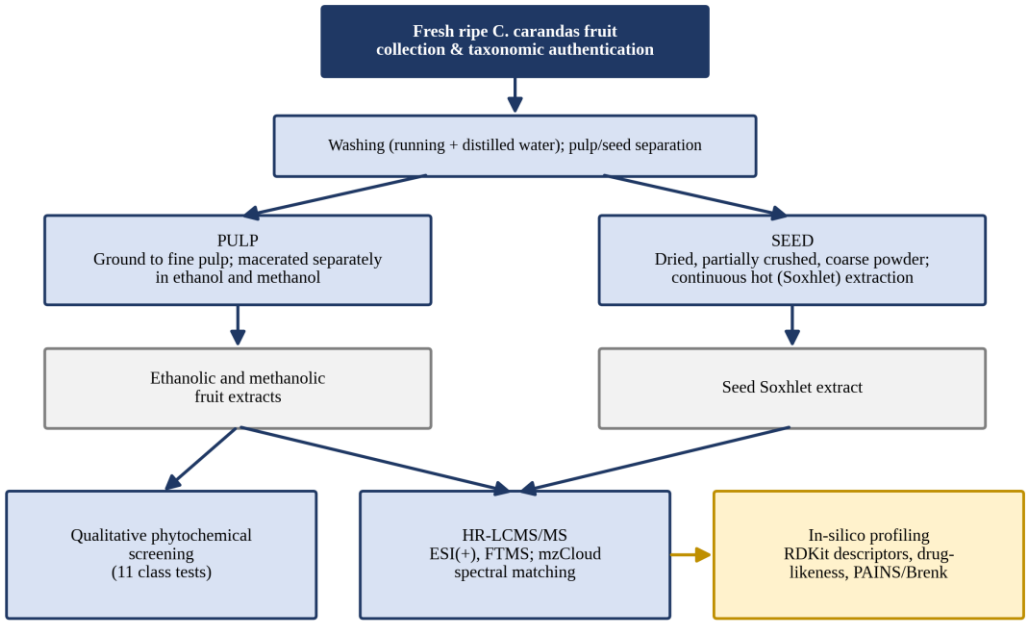


Fig. 1. Experimental workflow adopted for the phytochemical and bioprospecting profiling of Carissa carandas fruit.

2.4 Qualitative phytochemical screening

The fruit extract was screened for major classes of secondary and primary metabolites using standard colour and precipitation reactions [14–17]. Alkaloids were detected with Mayer's/Wagner's reagents (cream/reddish-brown precipitate); phenols and tannins with 5% ferric chloride (blue-black or greenish colour/precipitate); flavonoids by the Shinoda (magnesium–hydrochloric acid) test (pink to red colour); saponins by the froth test (foam persisting 10–15 min); cardiac glycosides by the Keller–Kiliani test (brown ring at the interface); terpenoids and steroids by the Salkowski test (reddish-brown colour at the interface); carbohydrates by Molisch's test; proteins by the biuret test; and amino acids by the ninhydrin test. Results were recorded as present (+), trace (±) or absent (−). These tests are presumptive; confirmatory identification was obtained by HR-LCMS/MS.

2.5 HR-LCMS/MS analysis

HR-LCMS/MS analysis was performed at the Centralised Instrumentation Centre (CIC), Swami Ramanand Teerth Marathwada University (SRTMU), Nanded, on a high-resolution Fourier-transform mass spectrometer (FTMS) coupled to liquid chromatography and operated in positive electrospray ionisation mode (ESI^+). Full-scan spectra were acquired over m/z 100–1200, and data-dependent MS^2 spectra were acquired by higher-energy collisional dissociation (HCD). Compound annotation was performed by accurate-mass formula matching and MS^2 spectral matching against the mzCloud library, and annotations were assigned confidence levels according to the Metabolomics Standards Initiative (MSI) [18] and the Schymanski scale [19].

2.6 In-silico physicochemical, pharmacokinetic and drug-likeness profiling

The structure of the annotated compound was encoded as a canonical SMILES string (COC(=O)c1cc2ccccc2cn1) and processed with the open-source RDKit cheminformatics toolkit (version 2026.03) [35]. Calculated descriptors comprised molecular weight, exact monoisotopic mass, Wildman–Crippen logP (WLOGP) and molar refractivity [20], topological polar surface area (TPSA) [21], hydrogen-bond donors and acceptors, rotatable bonds, aromatic ring count and fraction of sp^3 carbons (Fsp^3). Aqueous solubility was estimated by the ESOL model [22]. Drug-likeness was evaluated against the Lipinski [23], Veber [24], Ghose [25], Egan [26] and Muegge [27] rules; promiscuity and reactivity liabilities were screened with the PAINS [28] and Brenk [29] substructure filters; synthetic accessibility was scored by the Ertl–Schuffenhauer method [30]; and the quantitative estimate of drug-likeness (QED) was computed [31]. A bioavailability radar was constructed following the six-property framework of SwissADME [32]. Theoretical isotope distributions for the protonated molecule were calculated from IUPAC natural isotopic abundances, and mass error was calculated as $(m_{observed} - m_{theoretical})/m_{theoretical} \times 10^6$.

2.7 Adduct-family analysis of the full-scan spectrum

Labelled ions in the full-scan spectrum were tested for membership of a common adduct family by back-calculating the neutral mass (M) implied by each common ESI^+ adduct ($[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$ and the corresponding $[2M+X]^+$ dimers) using exact adduct masses, and retaining assignments whose implied M agreed within ±0.01 Da.

3. RESULTS

3.1 Sample processing and extraction

The authenticated fruits were processed into a fine, deep-red pulp that, on maceration, yielded intensely pigmented ethanolic and methanolic extracts (Fig. 2). The deep red-purple colour of both extracts is consistent with the anthocyanin-rich character reported for ripe karonda fruit [4,11]. The photographic record documents each processing stage from the fruiting shrub to the final extracts.

Fig. 2. Photographic documentation of Carissa carandas fruit processing: fruiting branch, fruits before and after washing, pulp ground in a mortar, pulp mixed separately with solvents, and the resulting ethanolic and methanolic extracts (original laboratory photographs).

3.2 Qualitative phytochemical profile

The fruit extract tested positive for nine of the eleven metabolite classes screened, namely alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, steroids and carbohydrates, whereas proteins and amino acids were detected only in trace amounts (Table 1; Fig. 3). The positive alkaloid reaction is notable in the context of the nitrogen-containing heterocycle subsequently annotated by HR-LCMS/MS (Section 3.3).

Table 1. Qualitative phytochemical screening of Carissa carandas fruit extract.

Metabolite class	Test	Observation	Result
Alkaloids	Mayer's / Wagner's	Cream-coloured precipitate	+
Flavonoids	Shinoda (Mg–HCl)	Pink to red colour	+
Phenols	Ferric chloride	Blue-black / green colour	+
Tannins	Ferric chloride	Blue-black / greenish precipitate	+
Saponins	Froth test	Stable foam, 10–15 min	+
Glycosides	Keller–Kiliani	Brown ring at interface	+
Terpenoids	Salkowski	Reddish-brown interface	+
Steroids	Salkowski	Reddish-brown interface	+
Carbohydrates	Molisch's [confirm]	[to be inserted]	+
Proteins	Biuret [confirm]	[to be inserted]	±
Amino acids	Ninhydrin [confirm]	[to be inserted]	±

+, present; ±, trace.

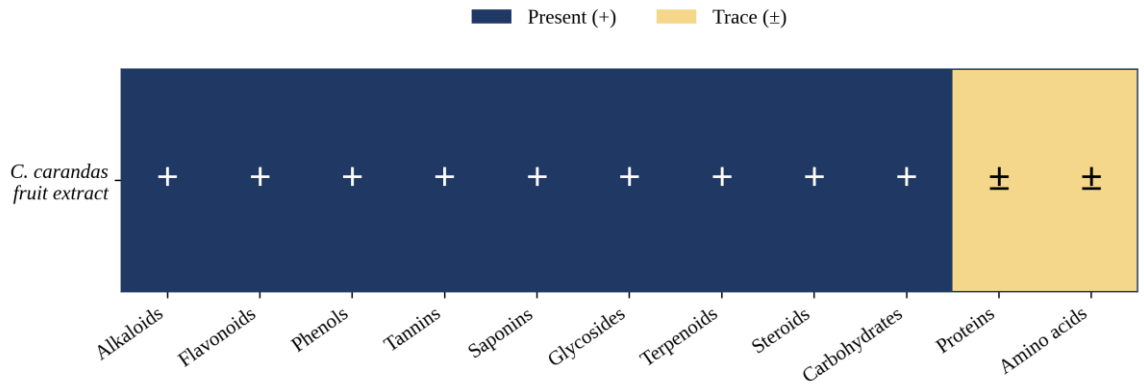


Fig. 3. Phytochemical class profile of Carissa carandas fruit extract (dark: present; light: trace).

3.3 HR-LCMS/MS annotation

Processing of the HR-LCMS/MS data (file CC(SLS), study CC(LHK)) annotated a chromatographic feature eluting at 5.076 min (maximum peak area 20,441,993) as methyl isoquinoline-3-carboxylate, $C_{11}H_9NO_2$ (Fig. 4; Table 2). The extracted-ion chromatogram showed a well-defined peak at 5.07 min with minor features at 4.29 and 6.14 min. The MS^2 spectrum of the precursor at m/z 188.0663 contained fragment ions at m/z 146.06 and 188.07 that matched the corresponding ions of the mzCloud reference spectrum in a mirror plot, yielding a best-match score of 76.0.

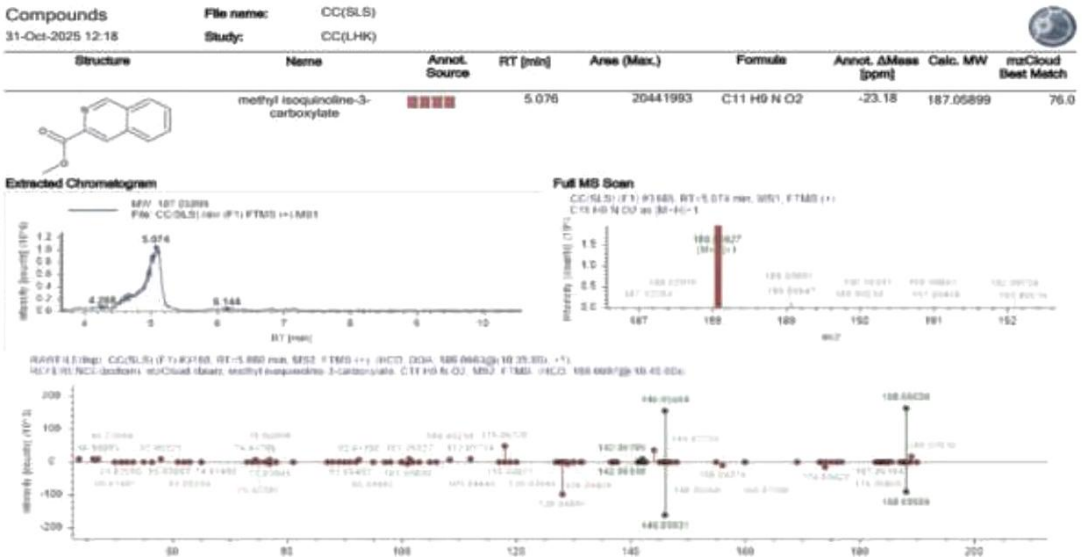


Fig. 4. HR-LCMS/MS annotation report for methyl isoquinoline-3-carboxylate: annotated structure and parameters, extracted-ion chromatogram (RT 5.074 min), full MS scan showing the $[M+H]^+$ ion, and MS^2 mirror plot of the sample spectrum (top) against .

Table 2. HR-LCMS/MS annotation parameters for the compound detected at RT 5.076 min.

Parameter	Value
Tentative identity	Methyl isoquinoline-3-carboxylate
Molecular formula	$C_{11}H_9NO_2$
Retention time (min)	5.076
Peak area (max.)	20,441,993
Theoretical monoisotopic mass (Da)	187.06333
Calculated (observed) mass (Da)	187.05899
Mass error (ppm)	−23.18
Precursor ion, observed $[M+H]^+$ (m/z)	188.0663
Precursor ion, theoretical $[M+H]^+$ (m/z)	188.07060
mzCloud best match	76.0
Ionisation / analyser	ESI^+ / FTMS, HCD MS^2

Parameter	Value
Identification confidence	MSI level 3 (Schymanski level 3)

Independent recalculation from the elemental formula reproduced the instrument-reported error exactly (−23.19 ppm; −4.3 mDa at m/z 188), and the theoretical isotope pattern of $[C_{11}H_{10}NO_2]^+$ predicts M+1 and M+2 abundances of 12.5% and 1.1%, respectively (Fig. 5). Because the mass error lies outside the ±5 ppm window customarily applied to FTMS data, the annotation is reported as a tentative structural class assignment (MSI level 3) rather than a putative identification.

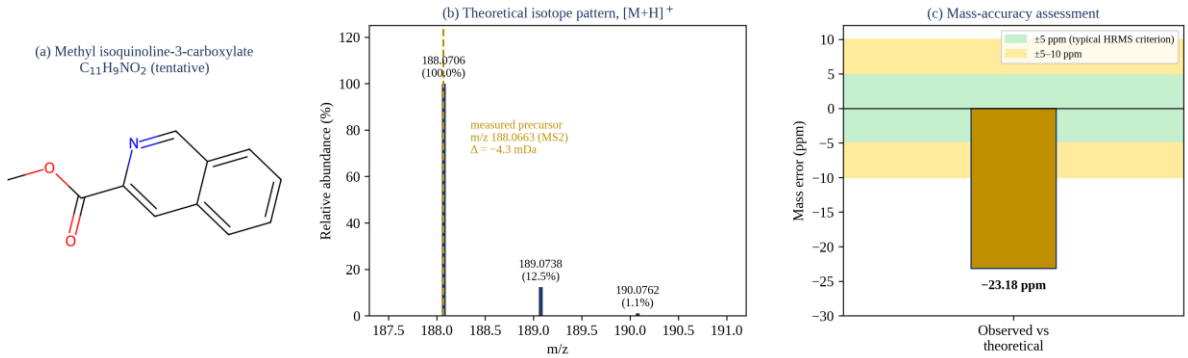


Fig. 5. (a) Structure of the tentatively annotated methyl isoquinoline-3-carboxylate; (b) theoretical isotope distribution of the protonated molecule with the measured MS^2 precursor; (c) observed mass error relative to conventional HRMS acceptance windows.

3.4 Full-scan profile and adduct-family analysis

The full-scan spectrum recorded at RT 12.24 min (scan #8245; normalisation level 2.51×10^7) was dominated by a base peak at m/z 415.20 and an intense ion at m/z 432.23 (≈52% relative abundance), with further labelled ions at m/z 171.15, 236.08, 316.31, 341.28, 453.16, 499.15, 548.26, 694.38, 795.46, 846.42, 867.35, 913.35, 988.95 and 1098.84 (Fig. 6).

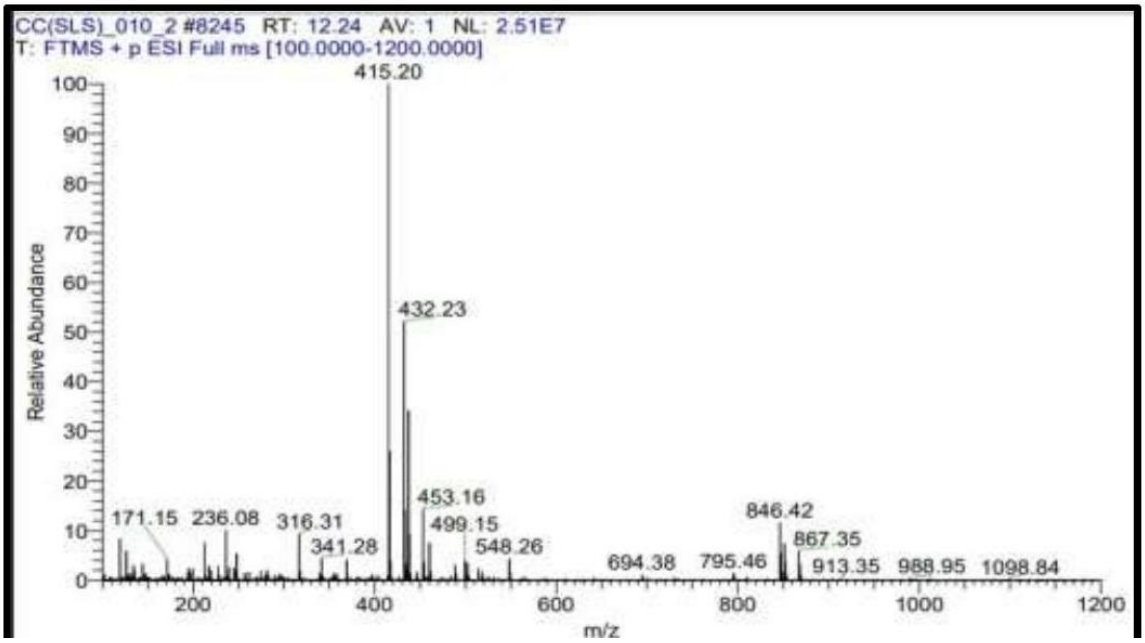


Fig. 6. Full-scan HR mass spectrum (FTMS, ESI^+, m/z 100–1200) of Carissa carandas extract at RT 12.24 min (original instrument output).

Adduct-family analysis showed that five of the labelled ions are explained by a single neutral species: m/z 415.20 ($[M+H]^+$), 432.23 ($[M+NH_4]^+$), 453.16 ($[M+K]^+$), 846.42 ($[2M+NH_4]^+$) and 867.35 ($[2M+K]^+$) each back-calculate to a neutral mass between 414.193 and 414.197 Da (mean 414.194 Da; Table 3; Fig. 7). The internal consistency of monomeric and dimeric adducts strongly suggests that these ions derive from one abundant constituent rather than from unrelated compounds. An unlabelled ion adjacent to m/z 432 in the printout lies where the corresponding $[M+Na]^+$ ion (theoretical m/z 437.18) would be expected.

Table 3. Assignment of full-scan ions (RT 12.24 min) to a common adduct family.

Observed m/z	Proposed ion	Adduct mass (Da)	Implied neutral M (Da)
415.20	$[M+H]^+$	1.00728	414.193
432.23	$[M+NH_4]^+$	18.03383	414.196
453.16	$[M+K]^+$	38.96316	414.197
846.42	$[2M+NH_4]^+$	18.03383	414.193
867.35	$[2M+K]^+$	38.96316	414.193
Mean ± SD	—	—	414.194 ± 0.002

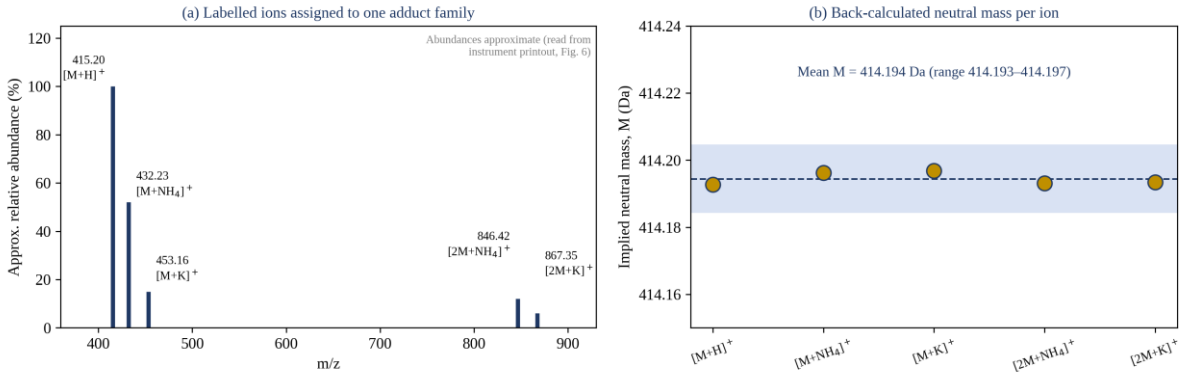


Fig. 7. Adduct-family analysis of the RT 12.24 min full-scan spectrum: (a) labelled ions assigned to one adduct family; (b) neutral masses back-calculated for each ion, converging on M ≈ 414.194 Da.

3.5 In-silico physicochemical and drug-likeness profile

The annotated alkaloid is a small (MW 187.20 Da; 14 heavy atoms), planar, predominantly aromatic molecule (two aromatic rings; Fsp^3 0.09) with moderate lipophilicity (WLOGP 2.02), low polar surface area (TPSA 39.19 $Å^2$), no hydrogen-bond donors, three acceptors and a single rotatable bond (Table 4). The ESOL model predicted good aqueous solubility (log S −2.74; ≈0.34 mg mL^{-1}). The compound satisfied the Lipinski, Veber, Ghose and Egan criteria without violation and incurred a single Muegge violation because its molecular weight is below 200 Da. No PAINS or Brenk alerts were raised, the synthetic accessibility score was 1.80 (very easy), and the QED was 0.64. In the bioavailability radar, five of six properties fell within the optimal range, the exception being saturation ($Fsp^3 < 0.25$), which reflects the flat heteroaromatic scaffold (Fig. 8).

Table 4. Calculated physicochemical, pharmacokinetic and drug-likeness descriptors of methyl isoquinoline-3-carboxylate.

Descriptor	Value	Reference range / criterion
Molecular weight (Da)	187.20	≤ 500 (Lipinski)
Heavy atoms / total atoms	14 / 23	20–70 total atoms (Ghose)
WLOGP (Wildman–Crippen)	2.02	≤ 5 (Lipinski); −0.4 to 5.6 (Ghose)
Molar refractivity	53.08	40–130 (Ghose)
TPSA ($Å^2$)	39.19	≤ 140 (Veber); ≤ 131.6 (Egan)
H-bond donors / acceptors	0 / 3	≤ 5 / ≤ 10 (Lipinski)
Rotatable bonds	1	≤ 10 (Veber)
Aromatic rings	2	—
Fsp^3	0.09	≥ 0.25 (radar optimum)
ESOL log S	−2.74 (soluble)	> −4 soluble
Lipinski / Veber / Ghose / Egan	0 / 0 / 0 / 0 violations	—
Muegge	1 violation (MW < 200)	MW 200–600
PAINS / Brenk alerts	0 / 0	—
Synthetic accessibility	1.80	1 (easy) – 10 (difficult)
QED	0.64	0–1 (higher = more drug-like)

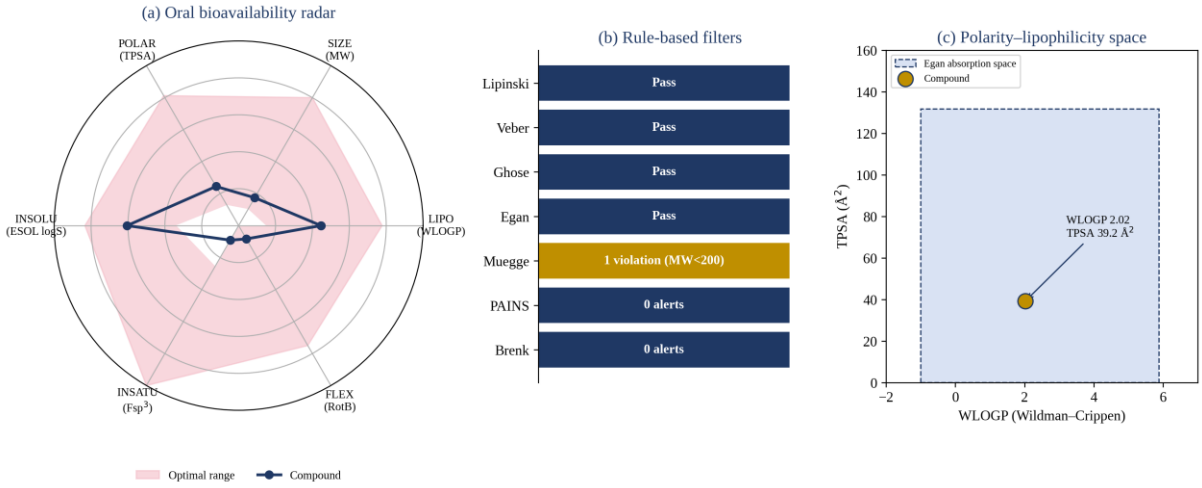


Fig. 8. In-silico profile of methyl isoquinoline-3-carboxylate: (a) oral bioavailability radar (shaded, optimal range); (b) compliance with rule-based drug-likeness and structural-alert filters; (c) position in WLOGP–TPSA space relative to the Egan absorption boundary.

4. DISCUSSION

4.1 Phytochemical composition

The qualitative profile of the fruit extract, rich in alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids and steroids, is concordant with previous reviews of C. carandas and the wider genus, in which triterpenes, cardiac glycosides, flavonoids and phenolics are recurrent constituents [3–5]. Flavonoids, phenolic acids and anthocyanins are the principal contributors to the antioxidant activity of karonda extracts [9,11,13], and the intense red-purple pigmentation of both alcoholic extracts in the present study is consistent with an anthocyanin-rich matrix. Tannins and saponins are frequently associated with antimicrobial and membrane-active effects, whereas triterpenoids such as carandinol have demonstrated cytotoxic potential [10]. The breadth of metabolite classes detected thus provides a chemical rationale for the diverse ethnomedicinal uses of the fruit and supports its selection for bioprospecting.

4.2 Significance and caveats of the isoquinoline annotation

Isoquinoline alkaloids constitute a large, pharmacologically important family, and the annotation of an isoquinoline-3-carboxylate is consistent with the positive alkaloid test. Nevertheless, three considerations temper its interpretation. First, the mass error of −23.18 ppm exceeds the tolerance normally accepted for Orbitrap/FTMS data, and alternative $C_{11}H_9NO_2$ isomers—for example, indole-3-acrylic acid, a widely occurring tryptophan-derived metabolite—share the same elemental composition and must be excluded by MS^2 and retention-time comparison with standards. Second, a mzCloud score of 76.0 indicates a good but not definitive spectral match. Third, methyl esters of carboxylic acids are well-documented artefacts of extraction with methanol, particularly under heating or acidic conditions [33,34]. Methyl isoquinoline-3-carboxylate could therefore originate from esterification of isoquinoline-3-carboxylic acid during methanolic maceration. Comparison of methanolic and ethanolic extracts (the latter would give the ethyl ester if an artefact were forming) provides a simple and elegant test that is available within the present experimental design.

4.3 A dominant constituent of neutral mass ≈414.19 Da

The coherent family of monomeric and dimeric adducts at RT 12.24 min indicates that a single constituent of neutral monoisotopic mass ≈414.194 Da is among the most abundant ionisable species in the extract. Formation of ammonium and potassium adducts alongside the protonated molecule, and of proton-bound dimers, is typical of oxygen-rich neutral natural products such as glycosides, terpenoids and lignans that lack strongly basic sites. Given the relatively high mass defect, a heteroatom-rich formula is more likely than a hydrocarbon-rich sterol. Its accurate-mass formula and MS^2 spectrum should be the immediate priority, as this constituent may account for a meaningful share of the extract's bioactivity.

4.4 Drug-likeness and bioprospecting implications

The annotated alkaloid combines low molecular weight, moderate lipophilicity and low polar surface area, placing it well inside the property space associated with good passive intestinal absorption [23,24,26]. The absence of PAINS and Brenk alerts reduces the likelihood of assay interference or intrinsic reactivity [28,29], and the low synthetic accessibility score indicates that the compound or analogues could be readily synthesised for structure–activity studies [30]. Its single Muegge violation (MW < 200 Da) and low Fsp^3 are characteristic of fragment-like heteroaromatics, suggesting that the scaffold is better regarded as a fragment or starting point for elaboration than as a finished lead. These predictions are hypothesis-generating only and require experimental confirmation.

4.5 Limitations and future perspectives

This study is limited by the qualitative nature of the phytochemical screening, the single HR-LCMS acquisition, the tentative (MSI level 3) annotation, and the absence of quantitative and cell-based bioactivity data. Future work will include quantitative estimation of phenolics, flavonoids and anthocyanins; validated antioxidant and antimicrobial assays; replicate HR-LCMS/MS acquisitions with blanks and authentic standards; accurate-mass

elucidation of the M ≈ 414.19 Da constituent; target-based molecular docking with validated protocols; and cytotoxicity and cell-cycle studies on cancer and normal cell lines.

5. CONCLUSION

Authenticated Carissa carandas fruit was processed through a reproducible extraction pipeline and shown to contain a broad spectrum of phytochemical classes, including alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids and steroids. HR-LCMS/MS tentatively annotated methyl isoquinoline-3-carboxylate and revealed a dominant, as-yet unannotated constituent of neutral mass ≈414.19 Da. In-silico profiling predicted favourable drug-likeness for the annotated alkaloid with no structural alerts. These results establish a chemically characterised foundation for the bioprospecting of C. carandas fruit and define clear priorities for compound confirmation, quantitative phytochemistry and cell-based bioactivity evaluation.

CRediT authorship contribution statement

Dhanashri Nagesh Pawar: Investigation, Methodology, Formal analysis, Visualisation, Writing – original draft.
L. H. Kamble: Conceptualisation, Supervision, Resources, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Raw HR-LCMS/MS files and all data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical statement

This study did not involve human participants or experimental animals.

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