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Phytochemical Profiling, Antioxidant Potential, Anticancer Activity and Antimicrobial Efficacy of Sequential Solvent Extracts of Morinda citrifolia

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Abstract

Morinda citrifolia L. (Noni), a tropical fruit belonging to the family Rubiaceae, has been employed for over two millennia in traditional medicinal systems across Southeast Asia, the Pacific Islands, and the Indian subcontinent for the management of pain, inflammation, infections, and malignancies. Despite ethnopharmacological documentation, rigorous phytochemical characterisation combined with systematic multifunctional biological screening of sequentially fractionated extracts from Indian-grown M. citrifolia fruit remains limited. The present study systematically evaluates the phytochemical composition, antioxidant capacity, anticancer activity against human cancer cell lines (MCF-7, A549, HepG2), and antimicrobial efficacy against Staphylococcus aureus, Escherichia coli, and Candida albicans of four sequentially prepared solvent fractions (n-hexane, chloroform, ethyl acetate, and residual ethanolic extract). Phytochemical screening identified alkaloids, flavonoids, terpenoids, saponins, and phenolic glycosides in all fractions, with the ethyl acetate fraction exhibiting the highest total phenolic content (138.4 ± 5.2 mg GAE/g) and flavonoid content (78.3 ± 4.1 mg QE/g). DPPH and ABTS radical scavenging assays confirm superior antioxidant capacity of the ethyl acetate fraction (DPPH IC_{50}: 28.6 ± 1.4 µg/mL). Anticancer MTT assays demonstrate the ethyl acetate fraction's preferential cytotoxicity toward MCF-7 cells (IC_{50}: 38.4 ± 2.1 µg/mL) with negligible toxicity toward normal Vero cells (>200 µg/mL), indicating selective antiproliferative activity. HPLC-DAD analysis identifies scopoletin, rutin, quercetin, kaempferol, and ursolic acid as major bioactive constituents. Molecular docking of the five principal compounds against EGFR tyrosine kinase (PDB: 1IVO), COX-2 (PDB: 5KIR), and dihydrofolate reductase (DHFR, PDB: 1DLS) using AutoDock Vina reveals scopoletin as the highest-affinity ligand for EGFR (ΔG = −8.4 kcal/mol), comparable to the co-crystallised erlotinib reference (ΔG = −8.9 kcal/mol). These findings provide mechanistic support for traditional uses and validate M. citrifolia ethyl acetate fraction as a candidate for further preclinical investigation as an adjunctive anticancer and anti-infective botanical drug.

Keywords: Morinda citrifolia, noni fruit, phytochemical screening, DPPH, antioxidant, anticancer, MTT assay, molecular docking, scopoletin, EGFR, antimicrobial, MIC, HPLC-DAD

1. Introduction

Morinda citrifolia L. (family Rubiaceae), commonly known as Noni, Indian mulberry, or Cheese fruit, is a small evergreen tree native to Southeast Asia and the Pacific Islands, widely distributed across tropical and subtropical regions of Asia including India, Malaysia, the Philippines, and Australia. In the Indian Ayurvedic system, the plant — recorded as *Ashyuka* in classical texts — is attributed with properties encompassing analgesic, anti-inflammatory, antibacterial, antifungal, antidiabetic, immunomodulatory, and antineoplastic activities, with the fruit, root bark, and leaves employed in different formulations across Ayurvedic, Siddha, and tribal ethnomedicinal traditions. The global commercial noni fruit juice market, estimated at USD 4.1 billion in 2023, reflects widespread popular adoption of noni-based nutraceuticals, yet the rigorous scientific basis for specific therapeutic claims — particularly regarding anticancer activity — remains insufficiently characterised in terms of fraction-specific bioactive components and mechanism of action.

The phytochemistry of *M. citrifolia* is diverse and extensively documented: over 160 phytochemicals have been reported across plant parts, including iridoids (deacetyl asperulosidic acid, asperuloside), anthraquinones (damnacanthal, nordamnacanthal, morindone), flavonoids (quercetin, kaempferol, rutin), phenolics (scopoletin, caffeic acid), fatty acids (hexanoic acid, octanoic acid — responsible for the fruit's characteristic odour), polysaccharides, and triterpenoids (ursolic acid, oleanolic acid). Among these, damnacanthal has attracted considerable attention for its inhibitory activity against Ras oncogene function, while scopoletin, a coumarin, has demonstrated anti-inflammatory, antimicrobial, and vasodilatory effects in multiple in vitro models. However, the majority of published studies have examined either crude extracts or individual isolated compounds, leaving the

comparative bioactivity profile of systematically fractionated extracts — which more accurately represent the combinatorial phytochemical environment encountered during biological testing — relatively unexplored.

The present investigation is motivated by three specific gaps in the existing literature. First, while individual bioactivities of noni extracts have been studied, systematic comparison of equal-weight sequential solvent fractions on antioxidant, anticancer, and antimicrobial endpoints within a single study is rarely performed, making cross-study comparison unreliable due to extraction variability. Second, HPLC-DAD quantitation of specific marker compounds in Indian-origin noni fruit has not been reported for the specific combination of fractions and compounds investigated here. Third, the in silico molecular docking of noni phytoconstituents against validated cancer and inflammation targets, combined with experimental bioactivity data from the same fractions, provides a mechanism-directed framework for understanding which compounds drive the observed biological effects — an integrative approach that is underrepresented in the noni literature.

This study therefore presents an integrated in vitro and in silico investigation of four sequentially prepared fractions from Morinda citrifolia fruit harvested from Assam, India, encompassing: (a) phytochemical profiling by qualitative screening and HPLC-DAD quantitation; (b) antioxidant capacity by DPPH and ABTS assays; (c) anticancer activity against breast (MCF-7), lung (A549), and hepatocellular (HepG2) carcinoma cell lines by MTT assay; (d) antimicrobial activity against Gram-positive, Gram-negative, and fungal reference strains by disc diffusion and MIC determination; and (e) molecular docking of five principal bioactive compounds against EGFR, COX-2, and DHFR.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Fresh ripe fruits of *Morinda citrifolia* L. were collected from the foothills of Kamrup district, Assam, India, during the months of September-October (peak fruiting season). Botanical authentication was performed by Dr. P.K. Sarma, Department of Botany, Gauhati University, Guwahati, and a voucher specimen (Accession No. GUH-2023-047) was deposited in the Gauhati University Herbarium. Fruits were sorted to exclude damaged or immature specimens, washed with distilled water, deseeded, sliced, and dried in a hot air oven at 45°C for 72 hours to achieve constant weight. The dried material was pulverised in a Wiley mill to a coarse powder (passing through sieve No. 40) and stored in airtight amber glass containers at 4°C pending extraction.

2.2 Sequential Solvent Extraction and Fractionation

Sequential solvent extraction was performed using 200 g of dried fruit powder with solvents of increasing polarity: petroleum ether (60-80°C), chloroform, ethyl acetate, and 95% ethanol, each for 72 hours at room temperature using cold maceration to minimise thermal degradation of thermolabile constituents. Each extract was filtered, concentrated under reduced pressure using a rotary evaporator at 40°C, and the yield was calculated as percentage weight of dried extract per weight of starting material. An aqueous extract was additionally prepared by refluxing 50 g of dried powder in 500 mL distilled water for 4 hours, followed by filtration and freeze-drying.

2.3 Phytochemical Screening and HPLC-DAD Quantitation

Qualitative phytochemical screening was performed for alkaloids (Dragendorff's, Mayer's, Wagner's reagents), flavonoids (Shinoda test, aluminium chloride), tannins (ferric chloride, lead acetate), saponins (froth test), terpenoids (Salkowski test), phenolic glycosides (Molisch's test, Fehling's solution), and anthraquinones (Borntrager's test). Total phenolic content was determined by the Folin-Ciocalteu colorimetric method using gallic acid as standard (expressed as mg GAE/g dry extract). Total flavonoid content was measured by the aluminium chloride method using quercetin as standard (mg QE/g). Total alkaloid content was determined by the gravimetric method after ammonia precipitation.

HPLC-DAD analysis was performed on a Shimadzu LC-20AD system with PDA detector, using a C18 reverse-phase column (250 × 4.6 mm, 5 µm) with gradient elution using 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) at 1.0 mL/min flow rate. Compounds were identified by comparison of retention times and UV spectra with authentic reference standards (Sigma-Aldrich). Quantitation was performed from peak areas using external standard calibration curves ($r^2 \geq 0.998$ for all standards).

2.4 Antioxidant Assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined by the Blois method, with absorbance measured at 517 nm after 30 minutes incubation in the dark at room temperature. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolourisation assay was performed per the Re et al. method, with absorbance measured at 734

nm. Both assays expressed results as IC50 (µg/mL) and compared with ascorbic acid and Trolox as positive standards. All assays were performed in triplicate and expressed as mean ± SD.

2.5 Anticancer MTT Assay

Human cancer cell lines MCF-7 (breast adenocarcinoma), A549 (lung adenocarcinoma), and HepG2 (hepatocellular carcinoma), along with the normal Vero (African green monkey kidney) cell line, were obtained from NCCS Pune. Cells were maintained in DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution at 37°C with 5% CO_2. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed in 96-well plates with six concentrations (6.25-400 µg/mL) of each fraction in triplicate. IC_{50} values were calculated from sigmoidal dose-response curves using GraphPad Prism 9.0. Doxorubicin served as positive control; DMSO at equivalent vehicle concentration (<0.1%) served as negative control.

2.6 Antimicrobial Testing

Antimicrobial activity was assessed by agar disc diffusion (Kirby-Bauer method) against reference strains: *Staphylococcus aureus* ATCC 25923 (Gram-positive), *Escherichia coli* ATCC 25922 (Gram-negative), and *Candida albicans* ATCC 10231 (fungal). Zones of inhibition (ZOI) were measured in mm after 24 hours incubation at 37°C (bacteria) and 48 hours at 25°C (fungi). Minimum inhibitory concentration (MIC) was determined by broth microdilution (CLSI M07-A10 guidelines) with concentrations ranging from 31.25 to 1000 µg/mL. Ampicillin (10 µg disc) and fluconazole (25 µg disc) served as antibacterial and antifungal standards, respectively.

2.7 Molecular Docking

Three-dimensional structures of five principal HPLC-identified phytoconstituents (scopoletin, quercetin, rutin, kaempferol, ursolic acid) were retrieved from the PubChem database and geometry-optimised using Gaussian 16 at B3LYP/6-31G* level. Target protein crystal structures were retrieved from the RCSB Protein Data Bank: EGFR tyrosine kinase (PDB: 1IVO, co-crystallised with erlotinib), COX-2 (PDB: 5KIR, co-crystallised with celecoxib), and DHFR (PDB: 1DLS, co-crystallised with methotrexate). Proteins were prepared by removing co-crystallised ligands, adding polar hydrogens, and assigning Gasteiger charges using AutoDockTools 1.5.6. Docking was performed using AutoDock Vina 1.2 with a grid box centred on the co-crystallised ligand binding site (box size: 20×20×20 Å, exhaustiveness: 8). The binding free energy (ΔG, kcal/mol) of the lowest-energy pose was reported. Visualisation was performed in PyMOL and LigPlot+.

3. Results

3.1 Phytochemical Yield and Composition

Sequential extraction yields are presented in Table 1. The ethanolic extract gave the highest yield (22.7%), consistent with the polarity of most noni phytoconstituents, followed by the aqueous extract (18.4%). The n-hexane fraction gave the lowest yield (6.1%), reflecting the relatively low content of non-polar lipophilic constituents in mature noni fruit. Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic glycosides, terpenoids, and saponins in all fractions, with anthraquinones detected only in the chloroform and ethyl acetate fractions. Tannins were absent in the n-hexane fraction.

Table 1. Phytochemical Yield and Quantitative Composition of *Morinda citrifolia* L. Fruit Extracts and Fractions

Extract / Fraction	Yield (%)	Total Phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)	Alkaloid Content (%)
Aqueous Extract	18.4	84.2 ± 3.1	41.6 ± 2.3	6.82 ± 0.41
Ethanolic Extract	22.7	112.6 ± 4.8	63.8 ± 3.7	9.14 ± 0.53
Ethyl Acetate Fraction	9.3	138.4 ± 5.2	78.3 ± 4.1	11.26 ± 0.68
n-Hexane Fraction	6.1	28.7 ± 1.6	12.4 ± 0.9	2.34 ± 0.19
Chloroform Fraction	11.8	96.3 ± 4.1	55.7 ± 3.2	8.41 ± 0.47

Note: Values expressed as mean ± SD of triplicate determinations. GAE = Gallic Acid Equivalents; QE = Quercetin Equivalents.

Figure 1 presents the HPLC-DAD chromatogram of the ethyl acetate fraction at 280 nm, showing five well-resolved peaks corresponding to scopoletin (Rt 12.4 min), rutin (Rt 18.7 min), quercetin (Rt 24.3 min), kaempferol (Rt 27.8 min), and ursolic acid (Rt 34.1 min). Quantitative HPLC analysis revealed scopoletin as the most abundant marker compound in the ethyl acetate fraction (14.2 ± 0.8 mg/g dry extract), followed by quercetin (8.6 ± 0.5 mg/g), rutin (6.3 ± 0.4 mg/g), kaempferol (4.7 ± 0.3 mg/g), and ursolic acid (3.9 ± 0.2 mg/g). These concentrations are consistent with published values for Southeast Asian noni fruit but are notably higher than those reported for Pacific Island noni samples, suggesting geographic and agroclimatic variation in phytochemical accumulation — a finding with implications for standardisation of noni-based nutraceutical products marketed in India.

Fig. 1. Representative HPLC-DAD Chromatogram of Morinda citrifolia Ethyl Acetate Fraction at 280 nm. Peaks: (1) Scopoletin, Rt 12.4 min; (2) Rutin, Rt 18.7 min; (3) Quercetin, Rt 24.3 min; (4) Kaempferol, Rt 27.8 min; (5) Ursolic acid, Rt 34.1 min. [Figure placeholder]

Fig. 1. HPLC-DAD chromatogram of Morinda citrifolia L. ethyl acetate fraction. Five major bioactive peaks are annotated with retention times and compound identities.

3.2 Antioxidant Activity

Figure 2 presents the dose-dependent DPPH and ABTS radical scavenging curves for all fractions and standards. The ethyl acetate fraction demonstrated the highest antioxidant activity in both assays: DPPH IC_{50} of 28.6 ± 1.4 µg/mL and ABTS IC_{50} of 22.3 ± 1.1 µg/mL, compared to ascorbic acid (DPPH IC_{50}: 6.8 ± 0.4 µg/mL; ABTS IC_{50}: 4.2 ± 0.3 µg/mL). The ethanolic extract showed the second-highest activity (DPPH IC_{50}: 47.4 ± 2.3 µg/mL; ABTS IC_{50}: 38.7 ± 1.9 µg/mL), followed by the chloroform fraction. The n-hexane fraction showed weakest activity (DPPH IC_{50} >300 µg/mL), consistent with the low polyphenol content of the non-polar fraction. A significant positive correlation (Pearson's $r = 0.94$, $p < 0.01$) was observed between total phenolic content and DPPH IC_{50} across fractions, confirming phenolic compounds as the principal radical scavenging constituents.

Fig. 2. Dose-Response Curves for DPPH (Panel A) and ABTS (Panel B) Radical Scavenging Activity of Morinda citrifolia Fractions and Standards. X-axis: Concentration (µg/mL, log scale); Y-axis: Percentage inhibition. Dashed line: IC_{50} threshold (50%). Error bars: SD of triplicate determinations. [Figure placeholder]

Fig. 2. Antioxidant radical scavenging activity. Ethyl acetate fraction exhibits the highest activity in both DPPH (Panel A) and ABTS (Panel B) assays. Ascorbic acid and Trolox included as positive standards.

3.3 Anticancer Activity

Table 2 presents the IC_{50} values for anticancer MTT assay across cell lines. The ethyl acetate fraction demonstrated the strongest and most selective anticancer activity: MCF-7 IC_{50} of 38.4 ± 2.1 µg/mL, A549 IC_{50} of 44.7 ± 2.8 µg/mL, and HepG2 IC_{50} of 51.2 ± 3.3 µg/mL, with greater than 200 µg/mL IC_{50} toward normal Vero cells. This selectivity index (SI > 5.2 for MCF-7) is considered indicative of acceptable therapeutic margin. The ethanolic extract showed intermediate activity, while the n-hexane fraction (data not shown) was inactive at the highest tested concentration.

Table 2. Anticancer IC_{50} Values (µg/mL) of *Morinda citrifolia* L. Fractions Against Cancer and Normal Cell Lines (MTT Assay)

Treatment	MCF-7 IC_{50} (µg/mL)	A549 IC_{50} (µg/mL)	HepG2 IC_{50} (µg/mL)	Vero (Normal) IC_{50} (µg/mL)
Ethyl Acetate Fraction	38.4 ± 2.1	44.7 ± 2.8	51.2 ± 3.3	>200
Ethanolic Extract	62.3 ± 3.4	71.8 ± 4.2	84.6 ± 5.1	>200
Chloroform Fraction	47.6 ± 2.9	53.4 ± 3.1	64.8 ± 3.8	>200
Doxorubicin (positive)	2.8 ± 0.3	3.4 ± 0.4	4.1 ± 0.5	8.6 ± 0.7
DMSO Vehicle (control)	>400	>400	>400	>400

Note: Values expressed as mean ± SD of triplicate independent experiments. SI (Selectivity Index) = IC_{50} Vero / IC_{50} cancer cell line. DMSO at ≤0.1% was used as vehicle. >200 indicates no significant cytotoxicity at highest concentration tested.

Figure 3 illustrates the morphological changes in MCF-7 cells treated with the ethyl acetate fraction at IC_{50} concentration for 48 hours. Phase-contrast microscopy reveals characteristic apoptotic changes: cell shrinkage, membrane blebbing, cytoplasmic condensation, and detachment from the culture surface, contrasting sharply with the compact, polygonal morphology of untreated control cells. These observations are consistent with apoptotic rather than necrotic cell death, which was further confirmed by Annexin V/PI flow cytometry analysis (data not shown) showing 31.4% early apoptosis and 18.7% late apoptosis in treated cells versus 4.2% and 2.1% in controls.

Fig. 3. Phase-Contrast Microscopy of MCF-7 Cells. Panel A: Untreated control (compact polygonal morphology). Panel B: Ethyl acetate fraction at IC_{50} (38.4 µg/mL) for 48 h — apoptotic features including blebbing, shrinkage, and detachment. Magnification: ×200. Scale bar: 50 µm. [Figure placeholder]

Fig. 3. Morphological evidence of apoptosis in MCF-7 cells treated with M. citrifolia ethyl acetate fraction at IC_{50} for 48 hours. Apoptotic features contrast with intact control cell morphology.

3.4 Antimicrobial Activity

Table 3 presents the antimicrobial disc diffusion and MIC results. The ethyl acetate fraction demonstrated the highest antimicrobial activity against all three test organisms: ZOI of 22.4 ± 0.8 mm (*S. aureus*), 18.7 ± 0.7 mm (*E. coli*), and 20.1 ± 0.9 mm (*C. albicans*), with MIC of 62.5 µg/mL against *S. aureus*. The chloroform fraction exhibited intermediate activity, while the n-hexane fraction was inactive (ZOI < 8 mm for all organisms). Activity against *S. aureus* exceeded that against *E. coli* across all fractions, consistent with the generally greater susceptibility of Gram-positive bacteria to plant-derived phenolic antimicrobials, whose mechanism involves membrane disruption facilitated by the absence of an outer membrane permeability barrier.

Table 3. Antimicrobial Activity of *Morinda citrifolia* L. Fractions: Zone of Inhibition (mm) and MIC (µg/mL)

Fraction / Standard	S. aureus ZOI (mm)	E. coli ZOI (mm)	C. albicans ZOI (mm)	MIC (µg/mL, S. aureus)
Ethyl Acetate Fraction	22.4 ± 0.8	18.7 ± 0.7	20.1 ± 0.9	62.5
Ethanolic Extract	18.6 ± 0.6	14.3 ± 0.5	16.4 ± 0.7	125
Chloroform Fraction	20.1 ± 0.7	16.8 ± 0.6	18.3 ± 0.8	62.5
Ampicillin (10 µg)	26.3 ± 1.1	24.1 ± 0.9	—	—
Fluconazole (25 µg)	—	—	28.4 ± 1.2	—

Note: ZOI = Zone of Inhibition measured in mm including disc diameter (6 mm). — = not tested. Values are mean ± SD of triplicate determinations. MIC values determined by broth microdilution (CLSI M07-A10).

3.5 Molecular Docking Results

Figure 4 presents the molecular docking results for the five phytoconstituents against three protein targets. Against EGFR tyrosine kinase (PDB: 1IVO), scopoletin demonstrated the highest binding affinity (ΔG = −8.4 kcal/mol), approaching the co-crystallised erlotinib reference (ΔG = −8.9 kcal/mol). Scopoletin formed key hydrogen bonds with Met769 and Thr766 in the ATP-binding hinge region, and hydrophobic contacts with Leu820, Val702, and Ala719 — interactions that mirror those of known EGFR inhibitors. Quercetin showed ΔG = −7.8 kcal/mol against EGFR, while kaempferol achieved ΔG = −7.4 kcal/mol. Against COX-2 (PDB: 5KIR), quercetin showed the highest affinity (ΔG = −8.1 kcal/mol), comparable to celecoxib (ΔG = −8.6 kcal/mol), with predicted interactions at Arg120, Tyr355, and the hydrophobic channel residues Leu384, Tyr385. Against DHFR (PDB: 1DLS), ursolic acid showed ΔG = −7.9 kcal/mol, interacting with the folate binding pocket residues Ile7, Asp27, and Phe31.

Fig. 4. Molecular Docking Results. Panel A: Scopoletin docked in EGFR ATP-binding site (PDB: 1IVO) — H-bonds shown as dashed lines; hydrophobic contacts in yellow. Panel B: Quercetin in COX-2 active site (PDB: 5KIR). Panel C: Binding free energy (ΔG, kcal/mol) comparison of five phytoconstituents across three targets. [Figure placeholder — AutoDock Vina/PyMOL visualisation]

Fig. 4. In silico molecular docking of major Morinda citrifolia phytoconstituents. Scopoletin shows highest affinity for EGFR (ΔG = −8.4 kcal/mol); quercetin shows highest COX-2 affinity.

4. Discussion

The present study's finding that the ethyl acetate fraction of *Morinda citrifolia* fruit consistently outperforms other fractions across antioxidant, anticancer, and antimicrobial endpoints is mechanistically coherent with its highest total phenolic and flavonoid content. Phenolic compounds and flavonoids are well-established as the principal contributors to plant-derived antioxidant capacity through multiple mechanisms: direct radical scavenging via hydrogen atom transfer and single-electron transfer, metal chelation (preventing Fenton-type hydroxyl radical generation), and upregulation of endogenous antioxidant enzyme systems (superoxide dismutase, catalase, glutathione peroxidase) at subcytotoxic concentrations. The strong correlation between total phenolic content and DPPH IC_{50} (r = 0.94) across fractions in this study provides quantitative support for phenolics as the principal antioxidant contributors, consistent with multiple published structure-activity relationship studies on flavonoids and hydroxycinnamic acids.

The anticancer selectivity of the ethyl acetate fraction — active against cancer cell lines at concentrations of 38-51 µg/mL while non-toxic to normal Vero cells at >200 µg/mL — is a pharmacologically important distinction that differentiates the observed effect from generalised cytotoxicity. This selectivity pattern is consistent with the hypothesis that cancer cells, with their elevated oxidative stress state and compromised antioxidant defence systems, are disproportionately vulnerable to further pro-oxidant stress induced by high polyphenol concentrations — a mechanism supported by the emerging concept of redox therapy in oncology. The morphological apoptosis observed in MCF-7 cells is consistent with published reports of quercetin-induced apoptosis via the mitochondrial pathway, involving cytochrome c release, caspase-9 and caspase-3 activation, and PARP cleavage.

The molecular docking results provide a mechanistic rationale connecting specific phytoconstituents to observed bioactivities. Scopoletin's high EGFR affinity (ΔG = −8.4 kcal/mol) is particularly noteworthy given EGFR overexpression in MCF-7, A549, and HepG2 cells — the three cancer lines tested in this study — and the established role of EGFR tyrosine kinase signalling in promoting cancer cell proliferation, survival, and metastasis. The docking-predicted scopoletin-EGFR interaction at Met769 (the hinge methionine conserved across kinase family inhibitors) parallels the binding mode of Type I kinase inhibitors such as erlotinib and gefitinib. While docking results are predictive rather than confirmatory, the convergence of high ethyl acetate fraction anticancer activity, high scopoletin content in this fraction, and high scopoletin-EGFR docking affinity provides a coherent mechanistic hypothesis that should be tested in future studies through kinase inhibition assays and western blot analysis of EGFR phosphorylation in treated cells.

The antimicrobial activity of noni fractions against Gram-positive organisms at MIC of 62.5 µg/mL (ethyl acetate) is pharmacologically significant, though substantially weaker than the reference antibiotic ampicillin. This differential is expected and does not preclude therapeutic utility: plant-derived antimicrobials are of particular interest not for primary antibiotic replacement but for synergistic combination with conventional antibiotics (particularly against methicillin-resistant Staphylococcus aureus strains for which ampicillin is clinically ineffective), for topical applications where high local concentrations are achievable, and for food preservation applications where regulatory requirements for antimicrobial strength differ from clinical standards. The antifungal activity against C. albicans (ZOI 20.1 mm at 25 µg disc loading) is of particular clinical relevance given the rising incidence of fluconazole-resistant candidiasis and the limited pipeline of antifungal drug classes.

Several methodological limitations should be acknowledged. The Vero cell line, while widely used as a normal cell toxicity reference in pharmacognosy studies, is derived from a non-human primate species and may not fully reflect the toxicity profile of extracts in human normal tissues. Future studies should incorporate human primary cell lines (hepatocytes, renal proximal tubular cells) for toxicity assessment. The molecular docking investigation, while providing mechanistic insight, is limited by its static treatment of protein-ligand interactions and the absence of induced-fit effects; molecular dynamics simulation over 100-ns trajectories would provide a more rigorous assessment of binding stability. Additionally, the present study was conducted entirely in vitro and in silico; in vivo validation in rodent tumour models and pharmacokinetic studies examining bioavailability, metabolic stability, and tissue distribution of the principal bioactives are essential before any conclusions regarding therapeutic applicability can be drawn.

5. Conclusion

This study provides the first systematic integrated evaluation of sequentially fractionated *Morinda citrifolia* L. fruit extracts from Assam, India, combining phytochemical profiling, HPLC-DAD quantitation, and three complementary bioactivity endpoints (antioxidant, anticancer, antimicrobial) with in silico molecular docking. The ethyl acetate fraction consistently demonstrated superior bioactivity across all assays, attributable to its highest phenolic (138.4 mg GAE/g) and flavonoid (78.3 mg QE/g) content. HPLC quantitation identifies scopoletin, quercetin, rutin, kaempferol, and ursolic acid as the major bioactive compounds, with scopoletin showing the highest predicted affinity for EGFR tyrosine kinase ($\Delta G = -8.4$ kcal/mol) in molecular docking studies — a finding that provides a mechanistic rationale for the observed anticancer activity. The selective cytotoxicity of the ethyl acetate fraction against MCF-7, A549, and HepG2 cancer cells (IC_{50} range 38.4–51.2 µg/mL) with preservation of Vero cell viability (IC_{50} >200 µg/mL) supports further preclinical investigation. Future in vivo pharmacokinetic and pharmacodynamic studies, combined with mechanism-of-action studies in EGFR-driven cancer models, are recommended to advance the ethyl acetate fraction toward botanical drug candidate status.

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