Phytochemical Profile and Bioactivity of *Acalypha indica* Aerial Methanolic Extract: Antioxidant, Antimicrobial, Anti-Inflammatory, and Anticancer Properties

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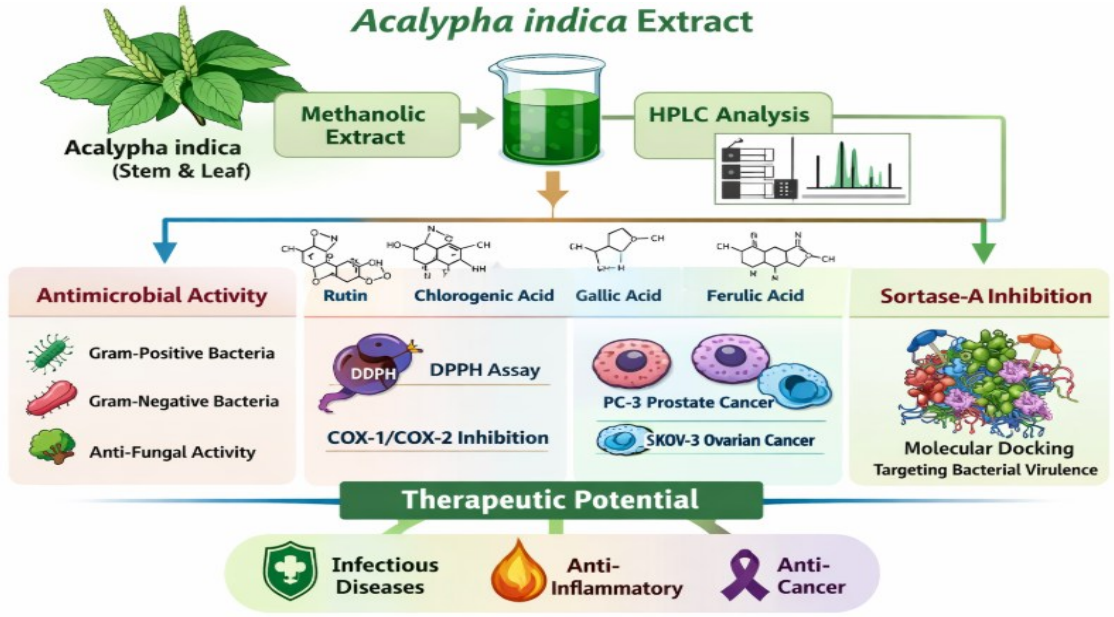
DOI: 10.15376/biores.21.2.4562-4579

GRAPHICAL ABSTRACT



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Acalypha indica, a plant used in traditional medicine, was evaluated for its phenolic composition and bioactivity. The methanolic extract of its aerial parts (stem and leaves) was analyzed using high-performance liquid chromatography (HPLC), identifying 17 phenolic compounds, including rutin (53.8 µg mL^{-1}), chlorogenic acid (53.3 µg mL^{-1}), gallic acid (36.3 µg mL^{-1}), and ferulic acid (33.3 µg mL^{-1}) as the primary constituents. These compounds correlated with the extract's antioxidant activity, confirmed by the DPPH radical-scavenging assay, yielding an IC_{50} of 6.8 µg mL^{-1}. The extract showed significant antimicrobial activity against Gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*, with inhibition zones exceeding that of Gentamycin. It also demonstrated moderate activity against Gram-negative bacteria, such as *Salmonella typhi* and *Klebsiella pneumoniae*, and antifungal activity against *Candida albicans*. Minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) assays showed bactericidal effects at 7.8 µg mL^{-1}. Additionally, the extract inhibited biofilm formation and hemolysin production, suggesting anti-virulence potential. The Cyclooxygenase (COX) inhibition assays indicated anti-inflammatory effects (IC_{50} = 11 µg mL^{-1}). Cytotoxicity tests on PC-3 prostate and SKOV-3 ovarian cancer cells revealed reductions in cell viability, with IC_{50} values of 11.52 and 10.31 µg mL^{-1}, highlighting *the therapeutic potential of Acalypha indica*.

DOI: 10.15376/biores.21.2.4562-4579

Keywords: Acalypha indica; Antioxidant activity; Antimicrobial properties; Cytotoxicity; Cancer therapy

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INTRODUCTION

The search for natural compounds with therapeutic potential has grown exponentially because of the increasing demand for alternative medicine and the rising concerns over the side effects and resistance associated with conventional drugs (WHO 2005a). One such plant, *Acalypha indica*, commonly known for its medicinal uses in traditional healing, is a promising candidate for further exploration in modern therapeutics (Chekuri *et al*. 2020). The methanolic extract of the aerial parts (stem and leaf) of *Acalypha indica* has been recognized for its diverse bioactive properties, yet its full chemical profile and pharmacological potential remain underexplored (Ravi *et al.* 2021). *Acalypha indica* has traditionally been used in herbal medicine to treat a variety of ailments; including infections, inflammation, cancer, and digestive issues (Labu *et al.* 2025). Its leaves and

stems are known to contain bioactive compounds that could offer broad-spectrum therapeutic benefits. The rich content of polyphenols in the plant, particularly flavonoids and phenolic acids, supports its potential as a source of natural compounds with multiple pharmacological actions (Chintada and Golla 2025).

The major active components identified in this plant include rutin, chlorogenic acid, gallic acid, ferulic acid, and quercetin; compounds well known for their antioxidant, anti-inflammatory, and antimicrobial effects (Kaurinovic and Vastag 2019). In particular, rutin and chlorogenic acid have been widely studied for their free radical-scavenging activity, while gallic acid and ferulic acid have demonstrated anti-inflammatory and antimicrobial effects in various studies (Singh *et al.* 2023). Additionally, the antibacterial and antifungal properties of *Acalypha indica* are well-documented, making it a potentially valuable candidate for combating antibiotic-resistant bacteria and fungal infections, which are increasing in prevalence globally (Angelini 2024).

The rising challenge of multidrug-resistant infections, chronic inflammatory diseases, and cancer has heightened the need for novel therapeutic agents that are both effective and safe. Natural plant extracts have become a focal point in the search for such agents due to their multi-target action and low toxicity profiles (Latif and Nawaz 2025). *Acalypha indica* offers a promising solution owing to its broad-spectrum bioactivity, which is not limited to microbial inhibition but also extends to modulation of immune responses, oxidative stress reduction, and cancer cell apoptosis (Dhama *et al.* 2014).

Molecular docking has been used to assess the binding affinities of bioactive compounds such as ellagic acid and chlorogenic acid against target proteins from *G. candidum* and *C. albicans*. Chlorogenic acid showed binding affinities of −5.70 kcal/mol with *C. albicans* (4YDE) and −7.84 kcal/mol with *G. candidum* (4ZZT), while ellagic acid exhibited affinities of −4.51 kcal/mol and −6.19 kcal/mol with the same targets (Rocha *et al.* 2022). Molecular docking is a computational technique to predict the interaction between a ligand and a target protein, providing insights into potential binding modes and affinities. While docking studies offer valuable preliminary data, the results should be considered theoretical and not fully reflective of biological conditions. To increase the reliability of docking predictions, experimental validation through in vitro assays is recommended.

This study investigates the phenolic composition of the methanolic extract from the aerial parts (stem and leaf) of *Acalypha indica* using high-performance liquid chromatography (HPLC) and evaluates its antioxidant, antimicrobial, anti-inflammatory, anti-biofilm, and anticancer activities. The primary aim was to identify and quantify the major bioactive compounds present in the extract, and to determine their potential therapeutic applications across multiple disease causing microorganisms. The research presented herein contributes to understanding how these natural compounds may work synergistically to offer a multi-target therapeutic approach, which is essential in addressing infectious diseases, chronic inflammation, and cancer. Given the increasing global burden of diseases associated with oxidative stress, infection, and inflammation, the need for novel therapies has never been more critical. This study not only provides an in-depth chemical analysis of *Acalypha indica* but also paves the way for its potential application in modern pharmacology and medicine. It offers new insights by presenting a comprehensive HPLC analysis of 17 phenolic compounds, with rutin identified as the major bioactive component. The findings from this study could lead to the development of new treatments derived from natural products, which are urgently needed to combat the rising tide of antimicrobial resistance, inflammatory diseases, and cancer.

EXPERIMENTAL

Materials

Acalypha indica mature plants were collected from the Abu Arish, Jazan region, Saudi Arabia from location with GPS coordinates 16°51'49.7"N 42°56'40.9"E, on 15th March 2025. The herbarium specimens are prepared with a voucher number (JAZUH 1642) and deposited in Jazan University Herbarium for further reference. The aerial parts (stem and leaves) of *Acalypha indica* were cleaned with distilled water, dried at 55 °C in an oven, and ground into a coarse powder. A total of 20 g of plant material was used for extraction, with methanol added at a solvent-to-plant ratio of 3:1 or 4:1. The mixture was left to macerate in a rotary shaker for 48 to 72 hours, then filtered through vacuum filtration. The methanolic extract was concentrated by evaporating the solvent under reduced pressure, and the final yield of the extract was 8.5% of the initial plant weight. The concentrated extract was stored in an airtight container for further analysis (Abada *et al.* 2025).

Methods

HPLC analysis of methanolic extract

The methanolic extract of *Acalypha indica* (20 µL) was mixed with 5 µL of 20% HCl and heated at 85 °C for 90 min. The final volume was adjusted to 100 µL with ethanol, and the solution was filtered through a 0.45 µm membrane. HPLC analysis was performed using an Agilent 1100 system equipped with a C18 column (4.6 mm × 250 mm, 5 µm particle size). The mobile phase for phenolic acids consisted of methanol and acetic acid/water (60:40), while flavonoids were separated using a 50:50 mixture of methanol and water (pH 2.8). The flow rate was set to 1.0 mL/min, and the detection wavelength was 280 nm. The column temperature was maintained at 30 °C. Retention times for the standards were as follows: rutin at 10.3 min, chlorogenic acid at 12.5 min, gallic acid at 8.2 min, and ferulic acid at 9.7 min under these conditions (Sahukari *et al.* 2021).

Antimicrobial activity and MIC detection

The antimicrobial activity of the methanolic extract was tested using the agar well diffusion method against bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Salmonella typhi* ATCC 6539, *K. pneumonia* ATCC 13883) and fungi (*Candida albicans* ATCC 10221 and *Aspergillus niger* ATCC 16888). A stock concentration of 50 mg of extract in 1.0 mL of 10% DMSO was prepared, and 100 µL of this stock solution was applied to each well or disc. The Minimum Inhibitory Concentration (MIC) was determined by the microdilution method with bacterial/yeast suspensions, incubated at 37 °C for 18 hours (bacteria) and 24 hours (fungi). The MIC was measured by optical density (OD) at 600 nm. Replicates of three wells were used for each concentration, and statistical analysis was performed using one-way ANOVA (Abada *et al.* 2025).

DPPH radical scavenging

Antioxidant activity of the methanolic extract was assessed by its ability to scavenge DPPH (2,2-Diphenyl-1-picrylhydrazyl) radicals. A 1.0 mL solution of 0.1 mM DPPH was mixed with 3 mL of the extract (1.95 to 1000 µg/mL), incubated for 30 min, and absorbance was measured at 517 nm. The IC_{50} was calculated from the dose–response curve, and ascorbic acid was used as a positive control (Selim *et al.* 2025)

Hemolysin activity and hemolysis assay

The hemolytic activity was evaluated by incubating bacterial supernatants with a 2% erythrocyte suspension. The mixture was centrifuged, and hemoglobin release was measured at 540 nm. Hemolysis was calculated as the percentage of hemoglobin release compared to control cultures (Thakur *et al.* 2016).

COX-1/COX-2 inhibition assay

COX-1 and COX-2 inhibition was assessed using the COX-1/COX-2 inhibitor screening assay kit. Test samples were dissolved in dimethyl sulfoxide (DMSO) (Merck, Germany) and tested at concentrations ranging from 1000 to 0.5 µg/mL. Celecoxib was used as a positive control. IC_{50} values were calculated using GraphPad PRISM (Redzicka *et al*. 2019).

Anti-biofilm activity

Biofilm formation was assessed using a microtiter plate assay. Bacteria were incubated with sub-MIC concentrations of the extract in 96-well plates. After staining with crystal violet, absorbance at 570 nm was measured to assess biofilm inhibition (El-Sayed *et al.* 2022).

Cytotoxicity assay

Cytotoxicity was assessed using the MTT assay on SKOV3 (human ovarian adenocarcinoma) and PC-3 (human prostate cancer) cell lines. Cells were treated with various concentrations of the methanolic extract, and after an exposure period of 24 to 72 h, the MTT solution was added and incubated for 4 h. Cell viability was then measured by absorbance at 620 nm, and the percentage of cell viability was calculated based on untreated controls. (Mashraqi *et al.* 2023).

Molecular docking study

Molecular docking of Rutin with Sortase A (PDB ID: 1T2P) was conducted using MOE (Molecular Operating Environment) docking. The protein was prepared by adding hydrogen atoms and assigning protonation states at pH 7.0. Rutin was selected for docking due to its highest concentration in the methanolic extract. Rutin's 3D structure was retrieved, energy-minimized, and conformational sampling was performed. The docking process used the Alpha Triangle algorithm, with the binding site defined around the catalytic triad. Top poses were analyzed for interactions and energies, and significant interactions were identified using MOE's ligand interaction module (Selvaraj *et al.* 2016).

RESULTS

Phenolic Composition of *Acalypha indica* Methanolic Extract

High-performance liquid chromatography (HPLC) of the methanolic extract from the aerial (stem + leaf) parts of *Acalypha indica* revealed seventeen phenolic/related constituents against external standards. Rutin was the most abundant analyte at 53.8 µg mL^{-1}, corresponding to 2.7 µg g^{-1} dry weight (DW), narrowly exceeding chlorogenic acid at 53.3 µg mL^{-1} (2.7 µg g^{-1} DW), thereby indicating a rutin-rich chemotype for this extract. Additional major constituents included gallic acid (36.3 µg mL^{-1}; 1.8 µg g^{-1} DW) and

ferulic acid (33.3 µg mL^{-1}; 1,7 µg g^{-1} DW), followed by intermediate levels of vanillin (21.4 µg mL^{-1}; 1.1 µg g^{-1} DW) and ellagic acid (17.0 µg mL^{-1}; 847.0 µg g^{-1} DW) (Fig. 1).

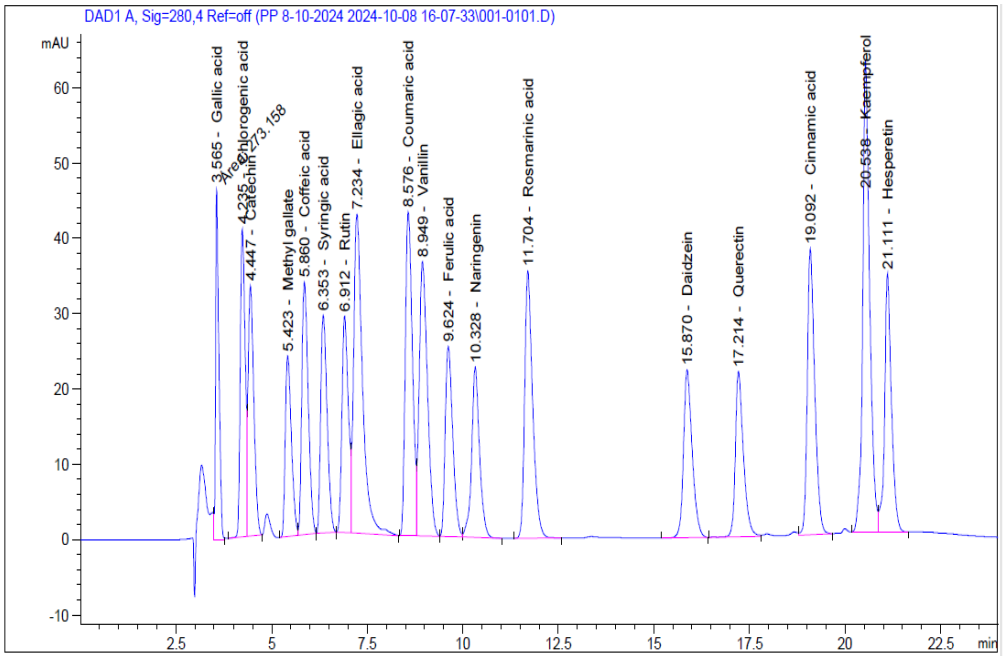

Fig. 1. HPLC chromatogram of the methanolic extract from the aerial (stem and leaf) parts of *Acalypha indica*. Major peaks represent the primary phytochemicals detected under the applied HPLC conditions.

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Minor constituents comprised syringic acid (6.0 µg mL^{-1}; 300.0 µg g^{-1} DW), rosmarinic acid (3.1 µg mL^{-1}; 156 µg g^{-1} DW), quercetin (2.8 µg mL^{-1}; 140.0 µg g^{-1} DW), naringenin (2.2 µg mL^{-1}; 108.0 µg g^{-1} DW), cinnamic acid (1.6 µg mL^{-1}; 81.2 µg g^{-1} DW), caffeic acid (1.0 µg mL^{-1}; 48.0 µg g^{-1} DW), daidzein (0.72 µg mL^{-1}; 36.0 µg g^{-1} DW), methyl gallate (0.63 µg mL^{-1}; 31.0 µg g^{-1} DW), and *p*-coumaric acid (0.42 µg mL^{-1}; 21.00 µg g^{-1} DW). Catechin, kaempferol, and hesperetin were not detected under the present conditions. Collectively, these results establish that the stem–leaf methanolic extract of *A. indica* is dominated by rutin, with chlorogenic, gallic, and ferulic acids as key secondary contributors, a profile that should guide subsequent correlations with antioxidant, antimicrobial, or other bioactivity readout.

Antibacterial and antifungal activities of Acalypha indica aerial parts

Antimicrobial activity of the *Acalypha indica* aerial methanolic extract was evaluated by disc diffusion using a stock prepared with 50 mg extract in 1.0 mL of 10% DMSO; antibiotic controls were gentamycin for bacteria and fluconazole for fungi at 1.0 mg mL^{-1}. Zone diameters (mm) are reported as mean ± SD. Against Gram-positive bacteria, the extract showed strong activity. *Bacillus subtilis* ATCC 6633 exhibited an inhibition zone of 40±0.6 mm, exceeding the gentamycin control (36±1 mm). *Staphylococcus aureus* ATCC 6538 showed 35 ± 0.5 mm versus 33±0.2 mm for gentamycin. These data indicate potency on par with—or slightly greater than—the reference antibiotic for both Gram-positive strains tested. Activity against Gram-negative bacteria was moderate to good. *Salmonella typhi* ATCC 6539 displayed a zone of 24±0.2 mm, matching gentamycin (24±0.1 mm) (Table 1).

Table 1. Antibacterial and Antifungal Activities of *Acalypha indica* Aerial Parts

Microorganism	Inhibition Zone	Antibiotic Control*
***Bacillus subtilis* (ATCC 6633)**	40 ± 0.6	36 ± 1
***Staph. aureus* (ATCC 6538)**	35 ± 0.5	33 ± 0.2
***K. pneumonia* (ATCC13883)**	21 ± 0.8	22 ± 0.4
***Salmonella typhi* (ATCC 6539)**	24 ± 0.2	24 ± 0.1
***Candida albicans* (ATCC 10221)**	27± 0.1	26 ± 0.1
***Aspergillus niger* (ATCC 16888)**	No effect	34 ± 0.1

* Control for Bacteria was Gentamycin and for fungi was fluconazole at concentration 1.0 mg/mL; Values represent measured inhibition zones indicating the extract's efficacy against microbial strains.

Klebsiella pneumoniae ATCC 13883 showed 21±0.8 mm compared with 22±0.4 mm for the control. Thus, the extract's effect was similar to that of the standard for *S. typhi* and was slightly lower for *K. pneumoniae*. In antifungal assays, the extract inhibited *Candida albicans* ATCC 10221 with a zone of 27±0.1 mm, comparable to (and marginally higher than) fluconazole (26±1 mm). By contrast, no detectable activity (NA) was observed against *Aspergillus niger* ATCC 16888 under the conditions employed, whereas fluconazole produced 34±1 mm.

Minimum inhibitory and minimum bactericidal concentrations

A primary stock was prepared by dissolving 10.0 mg of the *Acalypha indica* aerial methanolic extract in 10 mL sterile distilled water (1,000 µg/mL). Minimum inhibitory concentration (MIC) testing used a traditional twofold serial dilution series (1,000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8 µg/mL). Minimum bactericidal concentrations (MBCs) were read at/above the MIC by standard endpoint criteria. The *Acalypha indica* aerial methanolic extract showed strongest inhibitory and killing activity against Gram-positive bacteria and *Candida albicans*. Specifically, *Bacillus subtilis* (ATCC 6633) exhibited MIC = 7.8 µg/mL with MBC = 7.8 µg/mL (MBC/MIC = 1, bactericidal). *Staphylococcus aureus* (ATCC 6538) showed MIC = 7.8 µg/mL and MBC = 15.62 µg/mL (MBC/MIC ≈ 2, bactericidal) (Table 2).

Table 2. Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of *Acalypha indica* Extract

Microorganism	MIC (ug/mL)	MBC (ug/mL)
***Bacillus subtilis* (ATCC 6633)**	7.8	7.8
***Staph. aureus* (ATCC 6538)**	7.8	15.62
***K. pneumonia* (ATCC13883)**	62.5	125
***Salmonella typhi* (ATCC 6539)**	31.25	62.5
***Candida albicans* (ATCC 10221)**	15.62	15.62

Values indicate the lowest concentrations required to inhibit and kill the tested microbial strains.

Among Gram-negatives, *Klebsiella pneumoniae* (ATCC 13883) had MIC = 62.5 µg/mL and MBC = 125 µg/mL (MBC/MIC = 2, bactericidal), while *Salmonella typhi* (ATCC 6539) displayed MIC = 31.25 µg/mL and MBC = 62.5 µg/mL (MBC/MIC = 2, bactericidal). For *Candida albicans* (ATCC 10221), MIC = 15.62 µg/mL with MBC =

15.62 µg/mL (fungicidal equivalent). All MBC/MIC ratios ≤ 2 indicate bactericidal/fungicidal action across the tested organisms, with *B. subtilis* and *S. aureus* being the most susceptible, followed by *C. albicans*, and comparatively higher concentrations required for *S. typhi* and *K. pneumoniae*. These findings are consistent with the disc-diffusion profile and support follow-up time-kill and mechanism assays at sub-MIC to 2×MIC ranges.

Anti-biofilm activity by microtiter plate assay

Using the 96-well microtiter assay at sublethal exposures indexed to each strain's MBC (25%, 50%, and 75%), the methanolic aerial (stem + leaf) extract of *Acalypha indica* inhibited biofilm formation in a clear dose-dependent manner after 48 h at 37 °C. After crystal-violet staining and ethanol solubilization, A_{570} readings were converted to percent inhibition relative to untreated controls (methanol and medium blanks included). For *Bacillus subtilis* (ATCC 6633), mean inhibition reached 93.4 ±0.002% at 25% MBC, 94.78±0.006% at 50% MBC, and 98.47±0.006% at 75% MBC. *Staphylococcus aureus* (ATCC 6538) showed 91.9±0.002%, 92.5±0.002%, and 97.7±0.005% inhibition at 25%, 50%, and 75% MBC, respectively (Fig. 2).

Fig. 2. Anti-biofilm activity by microtiter plate assay

Among Gram-negative strains, *Salmonella Typhi* (ATCC 6539) exhibited 81.3±0.005% (25% MBC), 90.6±0.013% (50% MBC), and 96.0±0.003% (75% MBC), whereas *Klebsiella pneumoniae* (ATCC 13883) was less responsive at the lowest sub-MBC but converged at higher levels, with 64.3±0.009%, 85.7±0.003%, and 94.9±0.004% inhibition at 25%, 50%, and 75% MBC, respectively. Collectively, near-complete suppression (≈95 to 98%) was achieved for all organisms at 75% MBC, while Gram-positive bacteria were more sensitive at 25% MBC (≥ 92%–93%) than Gram-negative counterparts (64% to 81%). The very small standard deviations across triplicates indicate excellent assay precision.

Hemolysin inhibition assay

Hemolysin activity was quantified from culture supernatants of sub-MIC–treated bacteria (25%, 50%, 75% of each strain's MIC) *versus* untreated cultures. Cell densities were standardized (OD_{600} = 0.4), supernatants were incubated with a 2% erythrocyte suspension, and hemoglobin release was read at 540 nm. Percent hemolysis was calculated relative to 0.1% SDS (100% lysis, positive control) and unhemolyzed erythrocytes (negative control); data were obtained as triplicate means. Sub-MIC treatment markedly suppressed hemolysin-mediated RBC lysis across all tested organisms, with a clear dose–response (greater inhibition at 75% MIC). For *Staphylococcus aureus* (ATCC 6538), hemolysis fell to 6.3% at 25% MIC, 3.3% at 50% MIC, and 1.2% at 75% MIC, corresponding to 93.7%, 96.7%, and 98.8% inhibition, respectively (complete-lysis control (Fig. 3).

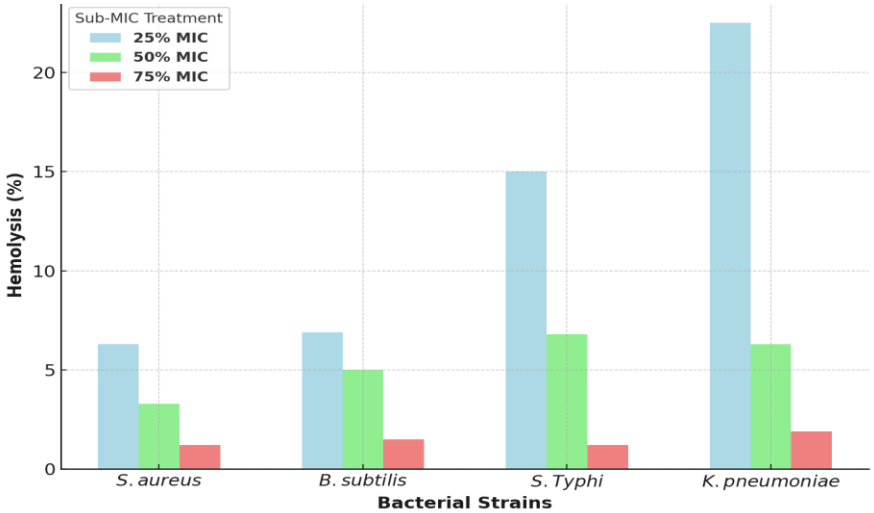


Fig. 3. Hemolysin activity of sub-MIC treatments on RBC lysis

For *Bacillus subtilis* (ATCC 6633), hemolysis measured 6.9% at 25% MIC, 5.0% at 50% MIC, and 1.5% at 75% MIC, which corresponds to 93.1%, 95.0%, and 98.5% inhibition. Among Gram-negative strains, *Salmonella Typhi* (ATCC 6539) showed 15.0% hemolysis at 25% MIC, 6.8% at 50% MIC, and 1.2% at 75% MIC, i.e., 85.0%, 93.2%, and 98.8% inhibition. *Klebsiella pneumoniae* (ATCC 13883) exhibited 22.5% hemolysis at 25%, 6.3% at 50% MIC, and 1.9% at 75% MIC, corresponding to 77.5%, 93.7%, and 98.1% inhibition.

DPPH radical-scavenging activity

Using the 0.1 mM DPPH assay (1 mL DPPH : 3 mL sample; 30 min at room temperature; 517 nm; triplicate reads), the methanolic extract of the aerial (stem + leaf) parts of *A. indica* showed a strong, monotonic dose-dependent increase in radical scavenging over 1.95 to 1000 µg mL^{-1}. Percent inhibition rose from 34.0% at 2.0 µg mL^{-1} to 42.5% (3.9 µg mL^{-1}), 51.6% (7.8 µg mL^{-1}), 59.6% (15.6 µg mL^{-1}), 67.9% (31.3 µg mL^{-1}), 75.9% (62.5 µg mL^{-1}), 83.3% (125 µg mL^{-1}), 89.3% (250 µg mL^{-1}), 92.4% (500 µg mL^{-1}), and reached 95.9% at 1000 µg mL^{-1}, indicating near-maximal quenching at the top dose (Fig. 4).

The IC_{50}, derived from the log-dose inhibition curve, was 6.8 µg mL^{-1}, confirming high antioxidant potency of this methanolic extract. Notably, >50% scavenging was already achieved at ~8 µg mL^{-1} (7.8 µg mL^{-1}: 51.6%), and the response approached a plateau from 250–1000 µg mL^{-1} (≈89%–96%). Assay performance was verified with the ascorbic acid reference, which produced 98.3% scavenging at 1000 µg mL^{-1} and an IC_{50} of 3.2 µg mL^{-1} under the same conditions. These controls validate the sensitivity and dynamic range of the DPPH readout used for the extract.

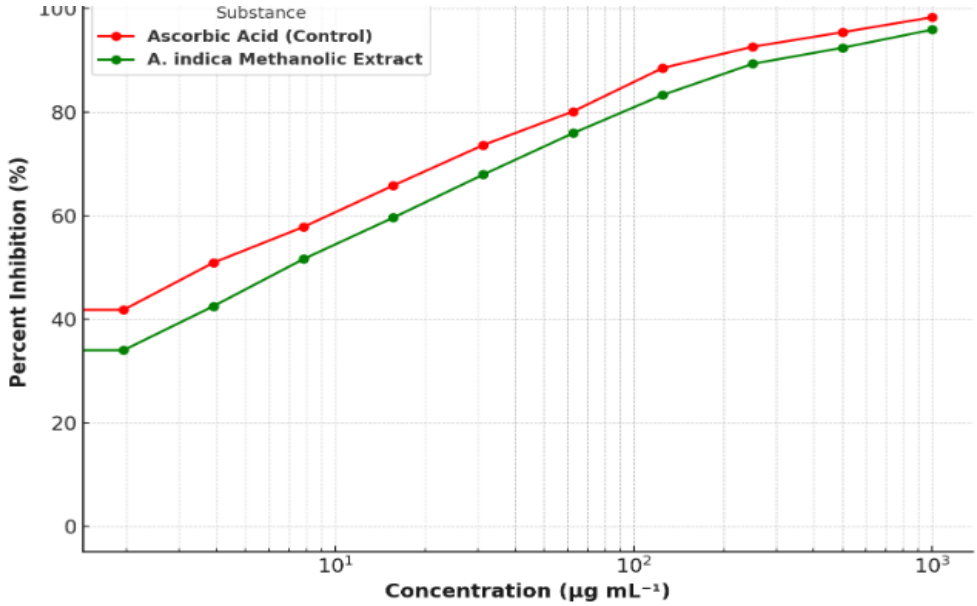


Fig. 4. DPPH Radical-scavenging activity of *A. indica* methanolic extract vs. ascorbic acid (Control)

In vitro COX-1/COX-2 inhibition

Using the Biovision screening kits (COX-1: K548; COX-2: K547), the test sample produced clear, concentration-dependent inhibition of both cyclooxygenase isoenzymes over 0.5–1000 µg mL^{-1} (1% DMSO vehicle; n=3 per dose), with celecoxib run in parallel as a reference inhibitor. For COX-1, the *Acalypha indica* aerial methanolic extract reached 93.9±0.1% inhibition at 1000 µg mL^{-1} and maintained a monotonic decline across the dilution series (*e.g.*, 72.9±0.4% at 62.5, 64.9±0.3% at 31.25, 49.6±0.9% at 7.8, and 11.9±0.1% at 0.5 µg mL^{-1}) (Fig. 5).

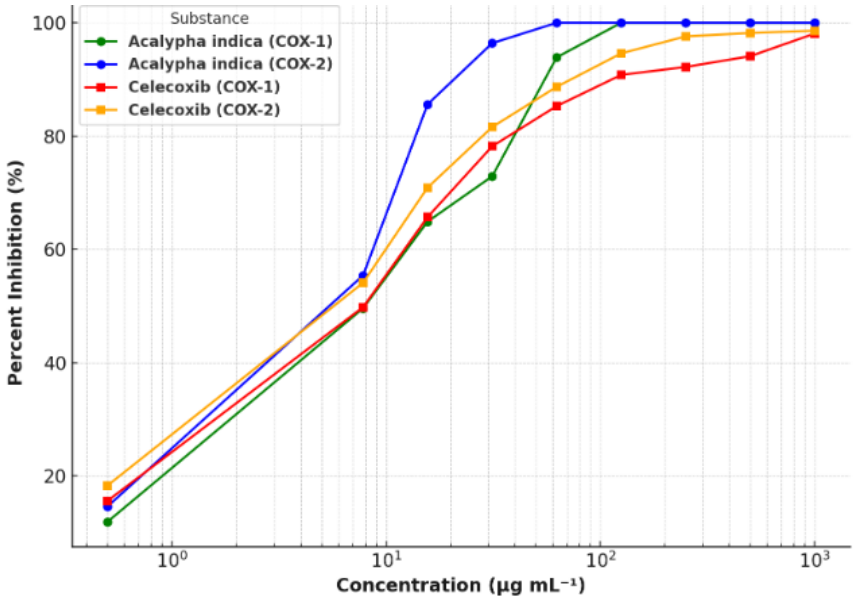


Fig. 5. *In vitro* COX-1 and COX-2 inhibition by *Acalypha indica* methanolic extract compared to Celecoxib as a reference

Nonlinear regression of the concentration–response yielded IC_{50} = 11.8±0.2 µg mL^{-1}. Under identical conditions, celecoxib showed 98.1±0.3% inhibition at 1000 µg mL^{-1} with IC_{50} = 7.4±0.2 µg mL^{-1} for COX-1. For COX-2, the *Acalypha indica* aerial methanolic extract achieved 96.4±0.6% inhibition at 1000 µg mL^{-1} and 85.6±0.8% at 250 µg mL^{-1}, decreasing to 55.4±0.3% at 15.6 and 14.6±0.3% at 0.5 µg mL^{-1}. The fitted IC_{50} was 11.22±0.2 µg mL^{-1}. Celecoxib, as expected for a COX-2–preferential inhibitor, was more potent in this assay (IC_{50} = 3.6±0.3 µg mL^{-1}; 98.6±0.1% inhibition at 1000 µg mL^{-1}). Taken together, the *Acalypha indica* aerial methanolic extract displayed low-two-digit µg mL^{-1} potency against both isoenzymes with minimal COX selectivity (COX-1/COX-2 IC_{50} ratio ≈ 1.05), whereas celecoxib showed greater COX-2 preference (ratio ≈ 2.1). These data indicated robust enzyme suppression at higher doses and sub-IC_{50} efficacy across the dilution range, supporting the anti-inflammatory potential of the *Acalypha indica* aerial methanolic extract and motivating fractionation to identify constituents responsible for dual COX inhibition.

Cytotoxicity on cancer cells using MTT assay

The methanolic extract of the aerial (stem + leaf) parts of Acalypha indica produced a concentration-dependent reduction in cell viability across PC-3 (prostate) and SKOV-3 (ovarian) cancer cell lines over the range 3.1 to 100 µg mL^{-1} (two-fold serial dilutions; n=3 per dose). Viability was calculated as (treated/control)×100 after 4 h MTT development and background subtraction at 620 nm. For PC-3, viability remained essentially unchanged at the two lowest concentrations (≈100% at 3.1 µg mL^{-1}; ≈98% at 6.25 µg mL^{-1}), and dropped sharply at 12.5 µg mL^{-1} to ~43%, indicating the onset of a steep cytotoxic transition (Fig. 6).

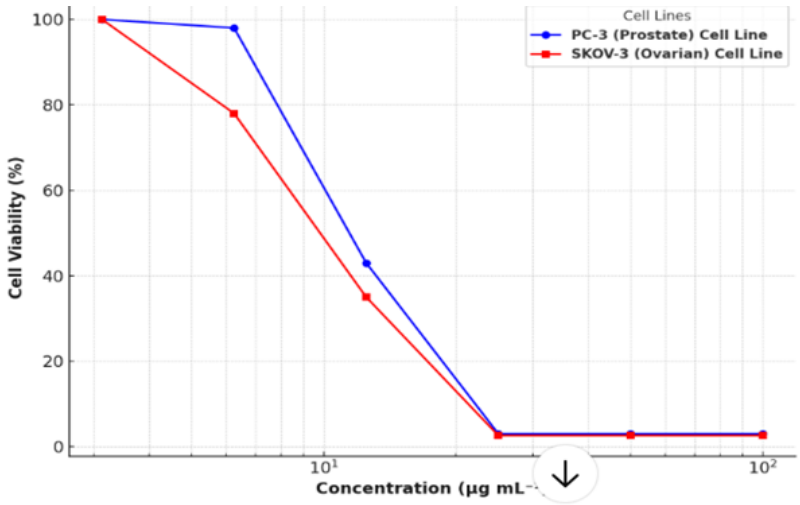


Fig. 6. Cytotoxicity of *Acalypha indica* methanolic extract on PC-3 and SKOV-3 cell lines

Near-complete loss of viability was observed from 25 µg mL^{-1} upward (~3% at 25 µg mL^{-1}; ~3% at 50–100 µg mL^{-1}), consistent with a maximal effect plateau at higher doses. Nonlinear regression of the sigmoidal dose–response yielded IC_{50} = 11.5 ± 0.20 µg mL^{-1}. For SKOV-3, the profile was similar but shifted slightly toward greater sensitivity. Viability was ~100% at 3.125 µg mL^{-1}, ~78% at 6.25 µg mL^{-1}, and ~35% at 12.5 µg mL^{-1}, followed by near-complete loss (~2.6%) at 25–100 µg mL^{-1}. The fitted IC_{50} = 10.31 ± 0.09 µg mL^{-1} confirms a modest left-shift relative to PC-3 (i.e., greater susceptibility of SKOV-3). Qualitative microscopic inspection during incubation aligned with these quantitative readouts: intact, confluent monolayers at ≤6.25 µg mL^{-1}; onset of rounding/shrinkage and partial monolayer loss around 12.5 µg mL^{-1}; and extensive detachment/granulation with near-total monolayer collapse at ≥25 µg mL^{-1}. These data demonstrate low–two-digit µg mL^{-1} potency of the extract against both lines, with ≥97% loss of viability at and above 25 µg mL^{-1} and a slightly lower IC_{50} for SKOV-3 than PC-3 under identical assay conditions.

Docking Study

Docking scores and energy

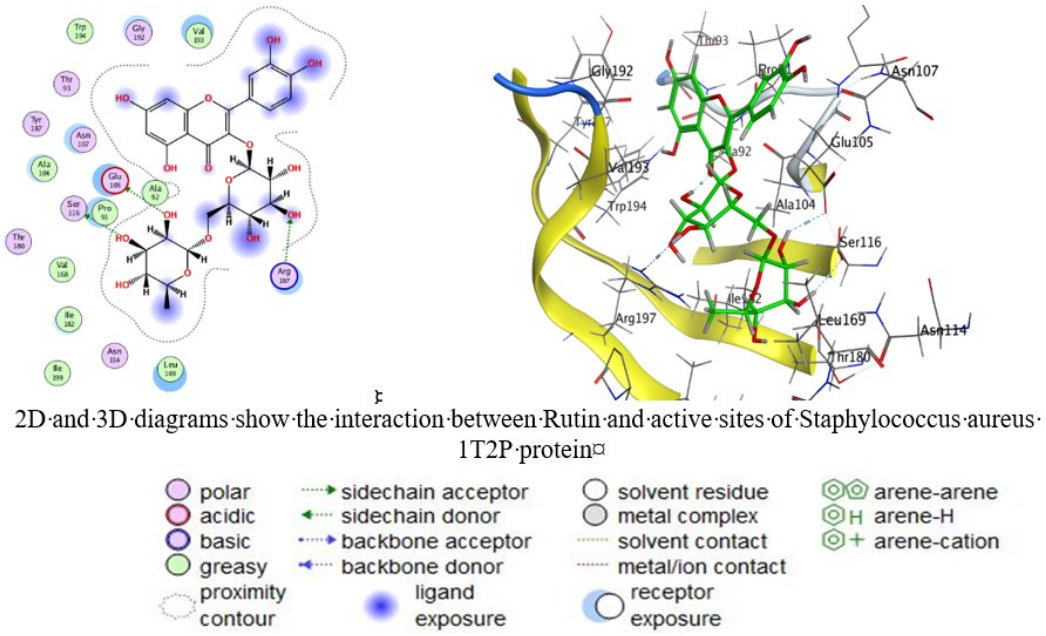
The best docking poses of rutin exhibited high binding affinity to Sortase−A, with docking scores (S) ranging from −7.09 to −6.94 kcal/mol (Table 3). The most negative energy pose (S = −7.09 kcal/mol) showed optimal binding stability, supported by favorable refinement energy (E_refine = −42.66 kcal/mol) and low root-mean-square deviation (rmsd_refine = 1.80 Å).

Table 3. Docking Scores and Energies of Rutin with Structure of Sortase-A from Staphylococcus aureus (PDB ID: 1T2P)

Mol	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
Rutin	-7.0916	1.7955089	-152.2038	-70.5703	-12.4458	-42.6641	-7.0916
Rutin	-7.07736	1.6477836	-145.1351	-85.1976	-13.4345	-41.143	-7.07736
Rutin	-7.05844	3.5669537	-137.7359	-89.4886	-11.9678	-39.937	-7.05844
Rutin	-6.96951	2.8000183	-289.2516	-57.8846	-11.5751	-39.2859	-6.96951
Rutin	-6.94496	2.16031	-138.6178	-65.2383	-11.4813	-40.09	-6.94496

Key interactions

Hydrogen bonds were identified with GLU105 (OE2, 2.87 Å, –1.5 kcal mol⁻¹), SER116 (OG, 3.05 Å, –1.1 kcal mol⁻¹), and ARG197 (NH1, 3.08 Å, –2.1 kcal mol⁻¹).



2D·and·3D·diagrams·show·the·interaction·between·Rutin·and·active·sites·of·Staphylococcus·aureus·1T2P·protein¤

Fig. 7. 2D and 3D diagrams showing interactions between Rutin and active sites of *S. aureus* 1T2P protein.

Table 4. Interaction of Rutin with Structure of Sortase A from *Staphylococcus aureus* (PDB ID: 1T2P)

Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Rutin	O 11	OE2 GLU 105 (A)	H-donor	2.87	-1.5
	O 13	OG SER 116 (A)	H-donor	3.05	-1.1
	O 6	NH1 ARG 197 (A)	H-acceptor	3.08	-2.1

These residues are part of Sortase-A's catalytic pocket, with ARG197 stabilizing the substrate-binding loop.

Binding pose visualization

2D/3D diagrams (Fig. 7) confirmed that rutin occupies the substrate-binding pocket.

DISCUSSION

The methanolic extract of the aerial parts (stem + leaf) of *Acalypha indica* displayed a promising and diverse range of bioactivities that could have significant therapeutic applications. This study provides detailed insight into the chemical composition and biological activity of the extract, focusing on its antimicrobial, antioxidant, anti-inflammatory, anti-biofilm, and anticancer properties (Ghosh *et al.* 2025). The findings suggest that the extract has potential as a multi-target agent, useful in the treatment of infections, inflammatory diseases, and cancer, primarily due to its rich content of flavonoids, phenolic acids, and other secondary metabolites (Namdeo *et al.* 2020).

Methanol was selected as the extractant due to its ability to efficiently dissolve a wide range of bioactive compounds, including phenolics and flavonoids, from plant materials. Its polarity and safety make it an ideal solvent for extracting bioactive metabolites in phytochemical studies. The phenolic profile identified in this study differs from previous research by quantifying 17 phenolic compounds, with rutin as the most abundant. Previous studies did not comprehensively assess the presence of compounds such as vanillin and ellagic acid. This expanded chemical profile offers new insights into *Acalypha indica*'s bioactive potential. Rutin being the most abundant (53.79 μg mL^{-1}), followed by chlorogenic acid (53.26 μg mL^{-1}), gallic acid (36.33 μg mL^{-1}), and ferulic acid (33.30 μg mL^{-1}). These compounds are well known for their antioxidant, anti-inflammatory, and antimicrobial properties, which likely contribute to the observed bioactivities. Rutin, a flavonoid glycoside, is especially noteworthy because of its potent antioxidant activity, which involves the scavenging of free radicals and the inhibition of oxidative stress (Alamami *et al.* 2023). This activity was corroborated by the DPPH radical-scavenging assay, where the extract demonstrated an IC_{50} value of 6.8 μg mL^{-1}, indicating strong antioxidant properties. Rutin's high concentration in the extract suggests that it plays a central role in the overall bioactivity, which is critical for diseases driven by oxidative damage, such as inflammatory disorders and cancer (Baliyan *et al.* 2022).

Chlorogenic acid, another key component, is known for its antioxidant, anti-inflammatory, and antimicrobial effects. Its high concentration further supports the extract's ability to neutralize free radicals and reduce oxidative stress, which are central to the pathogenesis of several diseases, including cancer, cardiovascular diseases, and diabetes (Huang *et al.* 2023). The presence of gallic acid (36.3 μg mL^{-1}) and ferulic acid (33.3 μg mL^{-1}) further contributes to the extract's anti-inflammatory and antimicrobial properties. Gallic acid is a known immune modulator and has been shown to induce apoptosis in cancer cells, while ferulic acid is recognized for its anti-inflammatory and antioxidant effects, contributing to the extract's ability to reduce inflammation and combat oxidative stress (Hadidi *et al*. 2024).

These findings suggest that the extract's phenolic composition plays a significant role in mediating multiple bioactivities and making it a versatile agent for therapeutic use (Sun and Shahrajabian 2023). The antimicrobial activity of the *Acalypha indica* methanolic extract was evaluated using disc diffusion and MIC methods. The extract exhibited strong antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus*

aureus), with inhibition zones of 40±0.6 mm and 35±0.5 mm, respectively, which were larger than the zones observed for the reference antibiotic, gentamycin (36±1 mm and 33±0.2 mm). These results indicate that the extract has a potent bactericidal effect against Gram-positive bacteria, which are often more susceptible to phenolic compounds due to their simpler cell wall structure (Suresh *et al.* 2021). The MIC values for *Bacillus subtilis* and *Staphylococcus aureus* were 7.8 µg mL^{-1}, and the MBC values were 7.8 µg mL^{-1} and 15.62 µg mL^{-1}, respectively, suggesting bactericidal activity. This is significant because the MBC/MIC ratio of 1.0 for *Bacillus subtilis* indicates that the extract is capable of killing the bacteria, while the MBC/MIC ratio for *Staphylococcus aureus* (≈2) indicates a slightly lower bactericidal effect but still shows strong antibacterial activity.

Against Gram-negative bacteria, the extract showed moderate to good activity, with inhibition zones of 24±0.2 mm for *Salmonella typhi* and 21±0.8 mm for *Klebsiella pneumoniae* (Suresh *et al.* 2021). The MIC values for *S. typhi* and *K. pneumoniae* were 31.2 µg mL^{-1} and 62.5 µg mL^{-1}, respectively. While the extract demonstrated effective antimicrobial activity against Gram-negative bacteria, its activity was slightly less potent than that observed against Gram-positive bacteria. The lower activity against Gram-negative bacteria can be attributed to their outer membrane, which acts as a barrier preventing many antimicrobial agents from entering the cell (Breijyeh *et al.* 2020). However, the extract still showed bactericidal activity, indicating its potential to treat a wide range of bacterial infections.

In antifungal testing, the extract showed significant inhibition of *Candida albicans*, with a zone of inhibition of 27±0.1 mm, which was comparable to fluconazole (26±1 mm), indicating its potential as an antifungal agent (Yadav and Gupta 2023). The lack of activity against *Aspergillus niger* could be due to differences in cell wall composition and the resistance mechanisms of filamentous fungi, which are often more difficult to treat with phenolic compounds (Shishodia *et al.* 2019). Biofilm formation is a major factor in the persistence of bacterial infections, especially in chronic conditions. The extract showed strong inhibition of biofilm formation in a dose-dependent manner. At the highest sub-lethal concentrations (75% MBC), the extract achieved up to 98% inhibition of biofilm formation across all bacterial strains tested, particularly in Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). This suggests that the extract may disrupt the early stages of biofilm formation, such as bacterial adhesion, extracellular polymeric substance (EPS) production, or quorum sensing, all of which are essential for biofilm development (Er-Rahmani *et al.* 2024). The hemolysin inhibition assay further highlighted the extract's anti-virulence potential. Hemolysins are toxins that cause damage to red blood cells and contribute to the virulence of many pathogens. The extract showed strong suppression of hemolysin production in both Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* and *Bacillus subtilis*, indicating that it can mitigate the toxic effects of bacterial infections (Duan *et al.* 2018). This anti-virulence action is beneficial because it reduces tissue damage and may enhance the effectiveness of conventional antibiotics. The COX-1/COX-2 inhibition assay revealed that the extract possesses significant anti-inflammatory activity, with IC_{50} values of 11.8 µg mL^{-1} for COX-1 and 11.2 µg mL^{-1} for COX-2.

These findings suggest that the extract could be a potential treatment for inflammatory conditions, as COX inhibition reduces the production of pro-inflammatory prostaglandins, which are involved in the pathogenesis of various diseases, including arthritis, cardiovascular diseases, and cancer (Bashir *et al.* 2024). The anticancer potential of the extract was demonstrated through its cytotoxicity against PC-3 prostate cancer and

SKOV-3 ovarian cancer cell lines. The extract exhibited dose-dependent reduction in cell viability, with IC_{50} values of 11.52 μg mL^{-1} for PC-3 cells and 10.31 μg mL^{-1} for SKOV-3 cells, suggesting that it has potent anticancer activity (Zhuang *et al.* 2022). The observed cytotoxicity could be attributed to the induction of apoptosis or cellular stress, which is characteristic of many phenolic compounds known to activate death pathways in cancer cells (Kuete *et al.* 2016). Additionally, molecular docking of rutin with Sortase-A reveals a novel antibacterial mechanism. The docking scores (−7.09 to −6.94 kcal/mol) suggest rutin's competitive binding potential with Sortase-A, similar to known inhibitors like curcumin derivatives. Key interactions include hydrogen bonds with ARG197, essential for substrate recognition, and GLU105 and SER116, critical for catalytic activity. Rutin's strong H-bond with ARG197 (−2.1 kcal/mol) indicates that it may mimic the LPXTG substrate motif. Compared to synthetic inhibitors like vinyl sulfones, rutin offers advantages in biocompatibility and low toxicity (Madkour *et al*. 2024).

CONCLUSIONS

1. The methanolic extract of *Acalypha indica* aerial parts (stem + leaf) demonstrated a wide range of bioactivities, including antimicrobial, antioxidant, anti-inflammatory, anti-biofilm, and anticancer effects, largely attributed to its phenolic composition, which includes high concentrations of rutin, chlorogenic acid, gallic acid, and ferulic acid.
2. The extract's multifaceted biological activity, particularly its strong binding to *S. aureus* Sortase-A, makes it a promising candidate for therapeutic use in infectious diseases and cancer therapy. Key interactions with GLU105, SER116, and ARG197 support rutin as a potential virulence inhibitor for developing anti-infectives.
3. Future studies should focus on bioassay-guided fractionation to identify the specific bioactive compounds responsible for these effects and explore their mechanisms of action in greater detail.

ACKNOWLEDGEMENT

We would like to disclose that AI-assisted language editing tools (*e.g*., Grammarly, DeepL Write) were used only to improve the language and clarity of the manuscript. All scientific content, data generation, simulations, analyses, and figures were produced entirely by the authors.

Conflict of Interest

Authors declare there is no conflict of interest.

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Article submitted: January 28, 2026; Peer review completed: March 15, 2026; Revised version received and accepted: March 23, 2026; Published: April 3, 2026.
DOI: 10.15376/biores.21.2.4562-4579