Antimicrobial Evaluation of *Carica papaya* Leaf Extract for Dermal Infections: Experimental and Computational Insights

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Abstract
Bacterial and fungal skin infections are commonly managed using antibiotic or antifungal therapy; however, the increasing concern over antimicrobial resistance has stimulated interest in plant-derived alternatives. This study evaluated the antimicrobial and antioxidant potential of *Carica papaya* leaf extract through integrated in vitro and in silico approaches.
Active-site molecular docking, skin-permeation prediction, and toxicological assessment were used to examine selected phytoconstituents and their possible interactions with microbial targets. The optimised ethanolic leaf extract, enriched in secondary metabolites, showed measurable activity against *Micrococcus luteus*, *Bacillus licheniformis*, *Rhizopus oryzae*, *Aspergillus niger*, and *Trichoderma spp*. Docking analysis identified favourable receptor-ligand interactions, while toxicity prediction indicated relatively low toxicity for several evaluated compounds, particularly ascorbic acid and papain. Collectively, the findings support the potential of *C. papaya* leaf extract as a promising source of bioactive constituents for topical antimicrobial applications.

Keywords: *Carica papaya*; antioxidant activity; antimicrobial activity; molecular docking; phytochemical analysis; dermal infection.

1. Introduction
For millennia, natural sources have served as a foundation for medicinal agents, and a significant proportion of modern therapeutics continues to be derived from traditional medicinal practices. Herbal extracts and plant-based formulations represent a longstanding scientific domain focused on the treatment of diverse ailments. Prior to the development and widespread adoption of synthetic drugs, healthcare systems relied primarily on medicines extracted from natural sources, predominantly of plant origin. With the rise of allopathic medicine, traditional remedies witnessed a decline in global popularity. However, growing awareness of unwanted side effects associated with chemical-based medications has reignited scientific and public interest in medicinal plants as sources of bioactive compounds for disease prevention and treatment [Tomlinson et al, 2015]. Despite thousands of years of documented use in the management of ailments ranging from skin conditions to cancer, scientific evidence supporting many applications of traditional plant-based therapeutics remains limited, and this critical gap needs to be addressed.
Although numerous phytochemicals have been identified for their biological activities, the therapeutic implications of using whole-plant preparations remain insufficiently understood. The phytochemical composition and potential pharmacological effects of many medicinal plants require systematic scientific investigation to ensure efficacy and safety.
Carica papaya (Papaya), belonging to the family *Caricaceae*, is a rapidly growing herbaceous plant indigenous to the tropical regions of the Americas, particularly southern Mexico and Central America. It is widely grown across tropical regions and can flower and fruit within a year of cultivation. The fruit is rich in pectin and contains β-cryptoxanthin, β-carotene-5-6-epoxide, lycopene, and zeta-carotene. Papaya is traditionally utilised to aid digestion, reduce inflammation, and support tissue healing [Singh et al 2020]. The present study investigates selected bacterial and fungal targets, particularly N-Acetylglucosamine, Glucan and Mannan subunits, based on their essential roles in maintaining cell-wall structural integrity. Inhibition of mannan and glucan-associated proteins disrupts the incorporation of glucose monomers linked via β-1,3 and β-1,6 glucan, leading to compromised membrane stability. This results in Ca^{2+} influx and subsequent saturation of calmodulin. The impaired formation of the calmodulin-calcineurin complex, which is critical for regulating fungal growth, cell-wall integrity, and stress responses, ultimately triggers fungal cell lysis [Callixte et al 2020]. In bacterial systems, particularly Gram-positive bacteria, the cell wall is composed of polysaccharide chains of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), extensively cross-linked with associated lipoteichoic acid. Enzymes involved in lipoteichoic acid synthesis are vital for maintaining membrane stability and peptidoglycan architecture; thus, targeting these proteins compromises bacterial cell-wall integrity and leads to cellular lysis [Baskaran et al 2012].

2. Material and Methods

2.1 *In silico* molecular docking

Three-dimensional structures (Figure 1) of the selected target proteins were obtained from the RCSB Protein Data Bank and prepared for docking by removing water molecules and heteroatoms, eliminating alternate conformations, adding polar hydrogens, and assigning Kollman charges. The target set comprised a β-glucan-associated fungal protein (PDB ID: 8IOX), lipoteichoic acid synthase (PDB ID: 2W5Q), a mannan-associated protein (PDB ID: 1OF4), and an N-acetylglucosamine-associated enzyme (PDB ID: 2CH5). The prepared protein structures were converted to PDBQT format using Python Molecular Viewer/AutoDockTools. Ligand structures and reference controls were retrieved from PubChem (Figure 2) and refined using LigPrep in Maestro 2015, followed by energy minimisation with the OPLS3 force field [Agarwal et al., 2020; Zengin et al., 2017].

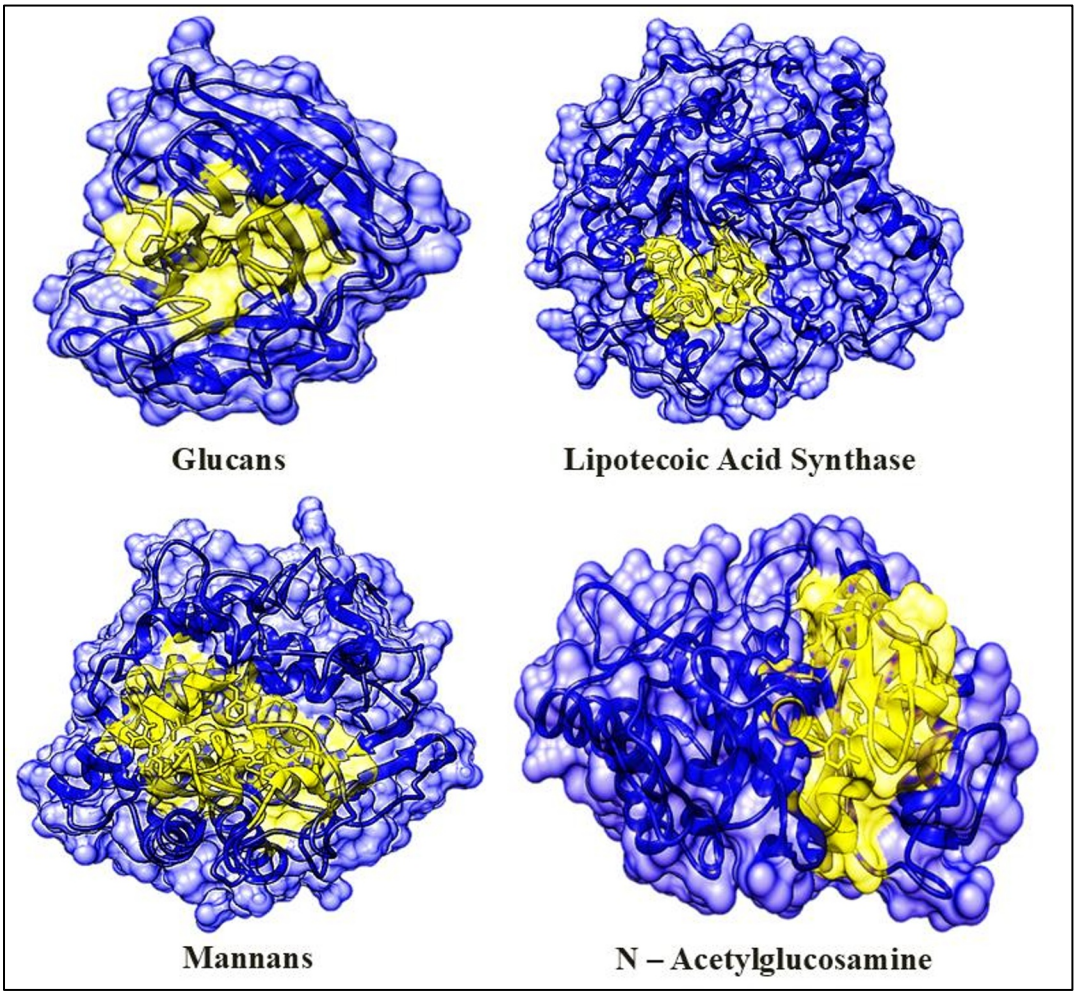

Figure 1. Three-dimensional visualisation of the selected receptor proteins retrieved from the RCSB Protein Data Bank, showing the predicted active-site pockets used for docking. Target proteins correspond to PDB IDs 8IOX, 2W5Q, 1OF4, and 2CH5.

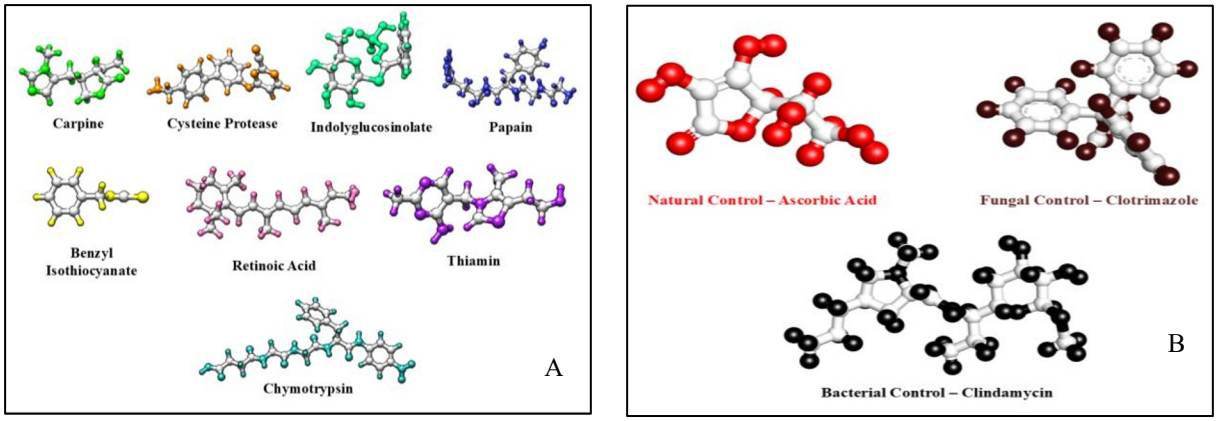

Figure 2. (A) Screened phytoconstituents and (B) reference control compounds retrieved from PubChem and visualised in UCSF Chimera.

Active-site pockets were identified using CASTp 3.0, and docking grids were generated in AutoDockTools 1.5.6. Molecular docking was then performed using AutoDock Vina. For each ligand-target pair, the lowest-energy pose and interaction pattern were examined. Docked complexes were visualised and interpreted using PyMOL, UCSF Chimera, and LIGPLOT+ [Hariyanti et al., 2018]. Because this study aimed to provide preliminary molecular support rather than definitive mechanistic proof, docking results were interpreted alongside the experimental antimicrobial findings and not as stand-alone evidence of biological efficacy. http://sts.bioe.uic.edu/castp/index.html?3trg

2.2 Toxicological Analysis

Toxicological prediction and dermal applicability assessment for selected phytoconstituents were carried out using ProTox-II. Predicted endpoints included organ toxicity, pathway-level biomarker responses, and LD50-based toxicity class. Skin-permeation estimates were also examined to support the discussion of possible topical applicability.
https://tox-new.charite.de/protox_II/

2.3 Antimicrobial activity

Antimicrobial activity was evaluated by the agar well diffusion method using ethanolic *C. papaya* leaf extract. The test organisms included *Micrococcus luteus*, *Bacillus licheniformis, Aspergillus niger, Trichoderma spp.*, and *Rhizopus oryzae.* Briefly, 100 µL of inoculum was spread uniformly onto nutrient agar plates for bacteria and potato dextrose agar plates for fungi. Wells of 6 mm diameter were prepared aseptically and filled with 200 µL of extract. Plates were incubated at 37 °C for bacterial strains and 25 °C for fungal strains, and the zones of inhibition were recorded after 48 h [Baskaran et al., 2012]. Ascorbic acid and water served as positive and negative controls, respectively.

2.4 Antioxidant Activity

Antioxidant activity was assessed by the DPPH radical-scavenging assay. A 1 mM DPPH solution was prepared in methanol, and ethanolic extract of papaya at concentrations ranging from 200 to 1000 µL/mL was tested. Absorbance was recorded at 517 nm against ethanol as blank, and percentage inhibition of the DPPH radical was calculated [Ang et al., 2012].

3. Results

3.1 In silico Molecular Docking Analysis

Docking analysis indicated that cysteine protease inhibitor (CPI) showed the strongest overall binding profile among the screened phytoconstituents, reaching a binding affinity of -8.4 kcal/mol against both the N-acetylglucosamine-associated enzyme and the mannan-associated target protein (Table 1). Chymotrypsin also showed favourable binding, particularly against bacterial targets. Overall, the results suggest that selected C. papaya phytoconstituents can interact favourably with proteins associated with microbial cell-wall integrity and related pathways.

Table 1. Predicted LD50 values and docking scores of screened phytoconstituents from *Carica papaya* against selected microbial target proteins.

Phytocompound	Predicted LD50 (mg/kg)	Toxicity class	Binding Affinity (K.Cal/ mol.)			
			Bacterial Targets		Fungal Targets	
			LTA synthase (2W5Q)	NAG-associated enzyme (2CH5)	Mannan-associated protein (1OF4)	β-glucan-associated protein (8IOX)
Papain (PAP)	2400	5	-6.7	-7.9	-7.1	-6.0
Cysteine protease inhibitor (CPI)	1000	4	-7.6	-8.4	-8.4	-6.7
Indolyglucosinolate (IG)	2000	4	-6.9	-7.5	-7.2	-6.8
Retinoic acid (RA)	1510	4	-6.9	-7.8	-6.4	-5.5
Carpine (CAR)	119	3	-5.2	-5.6	-5.3	-4.6
Thiamine (TH)	1923	4	-5.4	-5.5	-6.3	-5.2
Chymotrypsin (CHY)	3000	5	-7.8	-8.4	-7.8	-6.6
Benzyl isothiocyanate (BICT)	900	4	-4.6	-4.8	-4.9	-3.2
Natural control (AA)	3367	5	-5.8	-5.2	-6.1	-4.7
Bacterial control (CMY)	1095	4	-6.2	-6.6	-7.0	-5.6
Fungal control (CMZ)	708	4	-7.6	-7.2	-6.8	-5.2

Against the β-glucan-associated fungal target, CPI showed stronger predicted affinity than the natural and antifungal controls and formed hydrogen bonds with PHE27 and ARG26, together with several hydrophobic contacts that stabilised the docked complex (Figure 3A). CPI also displayed the highest binding affinity against the mannan-associated target (-8.4 kcal/mol), where the interaction pattern was dominated by hydrophobic contacts (Figure 3B). These findings suggest favourable accommodation of CPI within the predicted binding pockets of the fungal targets, while still remaining preliminary computational observations rather than proof of mechanism.

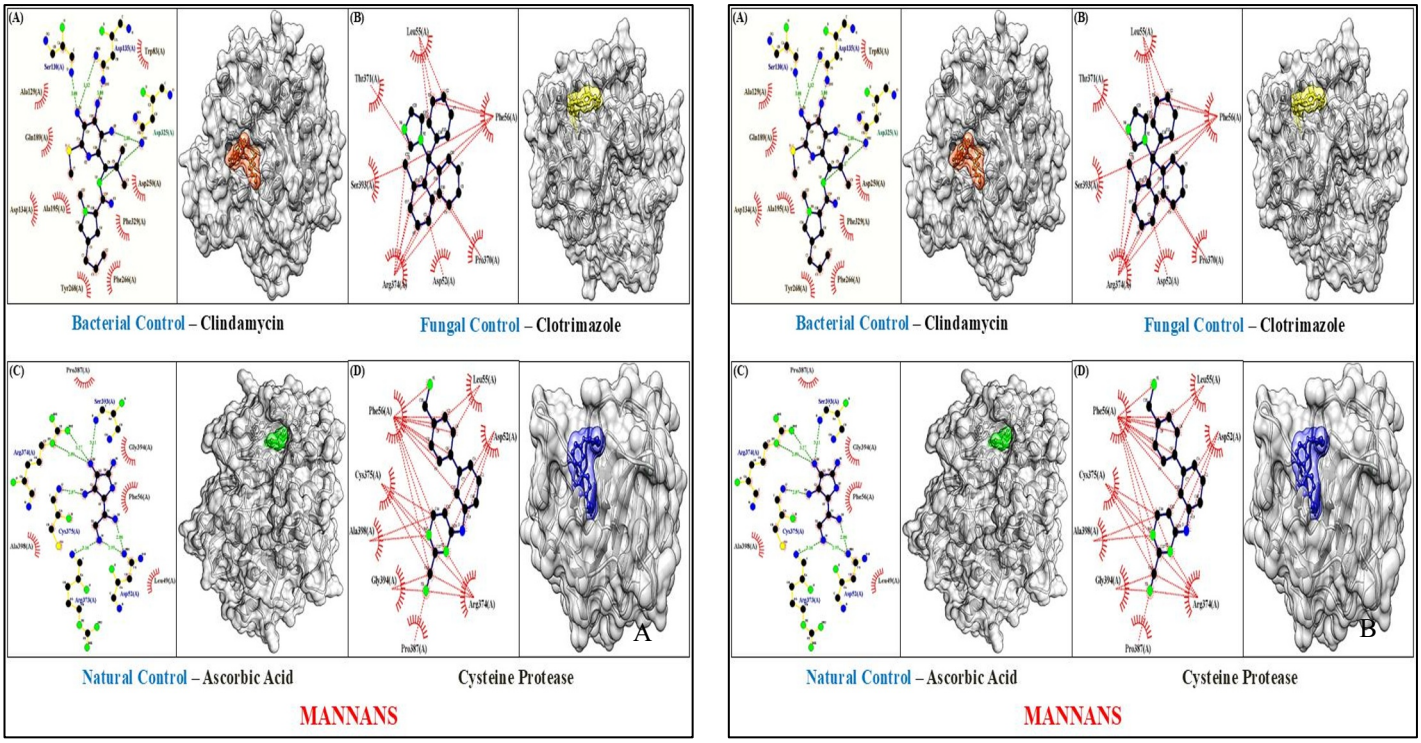

Figure 3. Receptor-ligand interaction profiles for the fungal targets: (A) β-glucan-associated protein (PDB ID: 8IOX) and (B) mannan-associated protein (PDB ID: 1OF4).

For bacterial targets, CPI also showed strong binding to lipoteichoic acid synthase and the N-acetylglucosamine-associated enzyme. The CPI-LTA synthase complex included a hydrogen bond with GLN304 and multiple hydrophobic interactions (Figure 4A), whereas the CPI-NAG-associated complex formed three hydrogen bonds with GLY108, TRP333, and ALA287 in addition to stabilising hydrophobic contacts (Figure 4B). Taken together, the docking results support the possibility that some phytoconstituents in C. papaya may interact with proteins relevant to bacterial cell-wall maintenance.

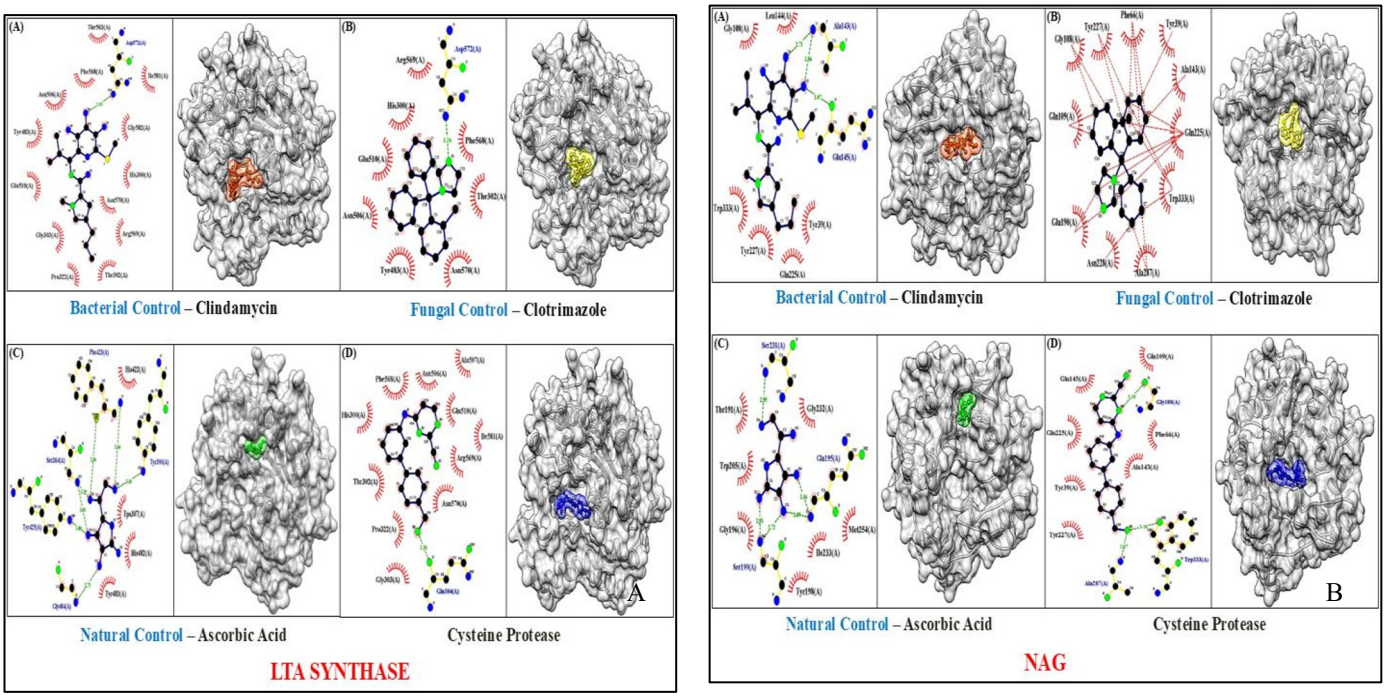

Figure 4. Receptor-ligand interaction profiles for the bacterial targets: (A) lipoteichoic acid synthase (PDB ID: 2W5Q) and (B) N-acetylglucosamine-associated enzyme (PDB ID: 2CH5).

3.2 Toxicological Assessment

Predicted toxicological assessment suggested an overall favourable safety profile for most screened phytoconstituents (Figure 5). Based on the reported LD50 classes, papain, cysteine protease inhibitor, indolyglucosinolate, retinoic acid, thiamine, benzyl isothiocyanate, and the study controls fell within low-to-moderate predicted toxicity classes, whereas carpine showed the least favourable toxicity class among the plant-derived compounds. Retinoic acid and benzyl isothiocyanate also showed relatively higher biomarker-associated toxicity signals than the other phytoconstituents.

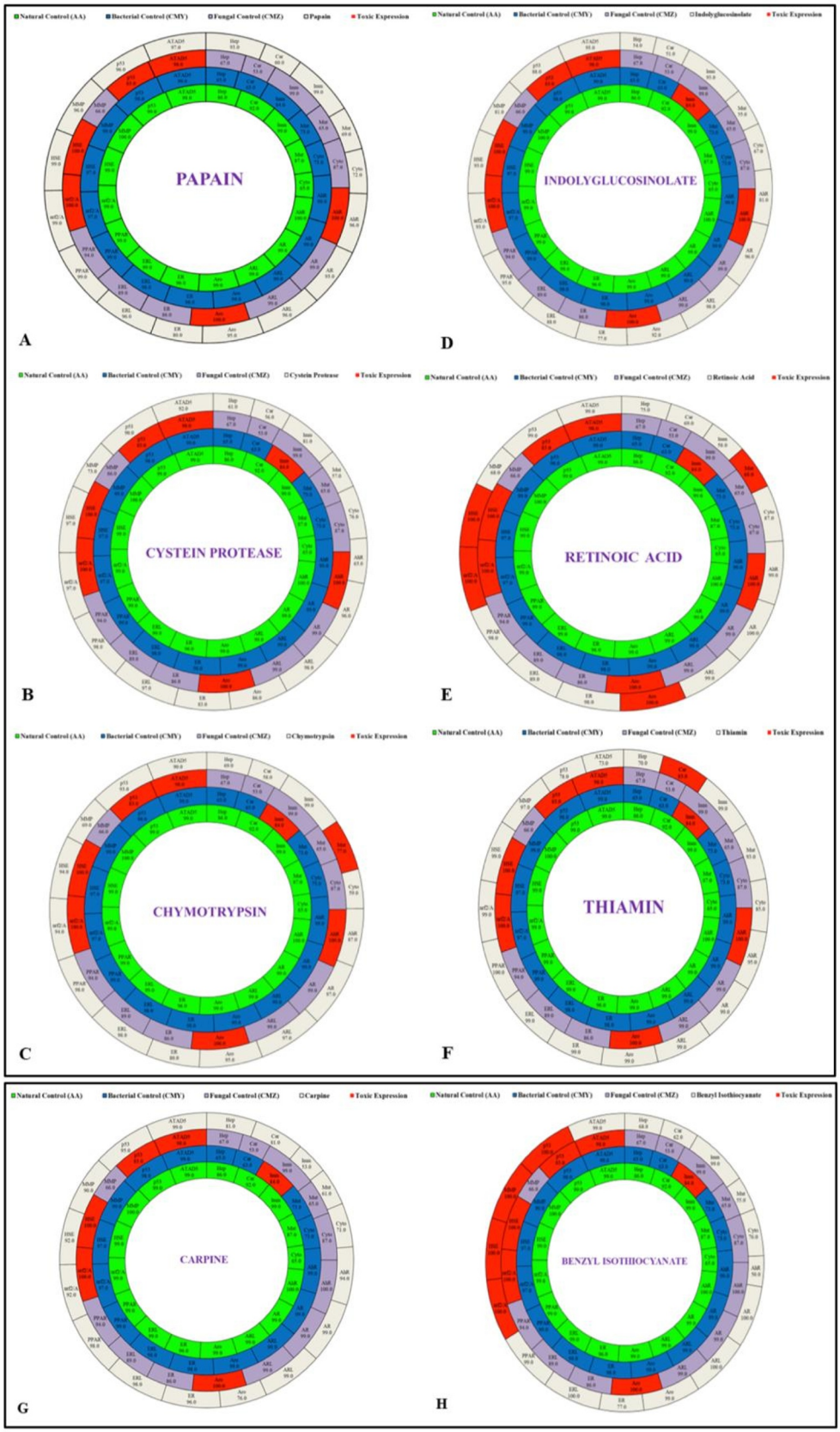

Figure 5. Comparative toxicological profiling of the screened phytoconstituents derived from Carica papaya extract.

Abbreviations: Hep: Hepatotoxicity; Car: Carcinogenicity; Imm: Immunotoxicity; Mut: Mutagenicity; Cyto: Cytotoxicity; AhR: Aryl Hydrocarbon Receptor; AR: Androgen Receptor; ARL: Androgen Receptor Ligand Binding Domain; Aro: Aromatase; ER: Estrogen Receptor Alpha; ERL: Estrogen Receptor Ligand Binding Domain; PPAR: Peroxisome Proliferator Activator Receptor Gamma; nrf2/A: Nuclear Factor (Erythroid – Derived 2) – like 2/Antioxidant Responsive Element; HSE: Heat Shock Factor

Response Element; MMP: Mitochondria Membrane Potential; p53: Phosphoprotein (Tumor Supressor); ATAD5: Atpase Family AAA Domain – Containing Protein 5.

3.3 Skin Permeation Rate

Because the intended application is dermal, skin permeation was considered an important supportive parameter (Figure 6). Papain showed the highest predicted permeation, whereas retinoic acid showed the lowest. Several phytoconstituents demonstrated permeation values comparable to, or higher than, the reference controls, supporting their potential relevance in topical formulations.

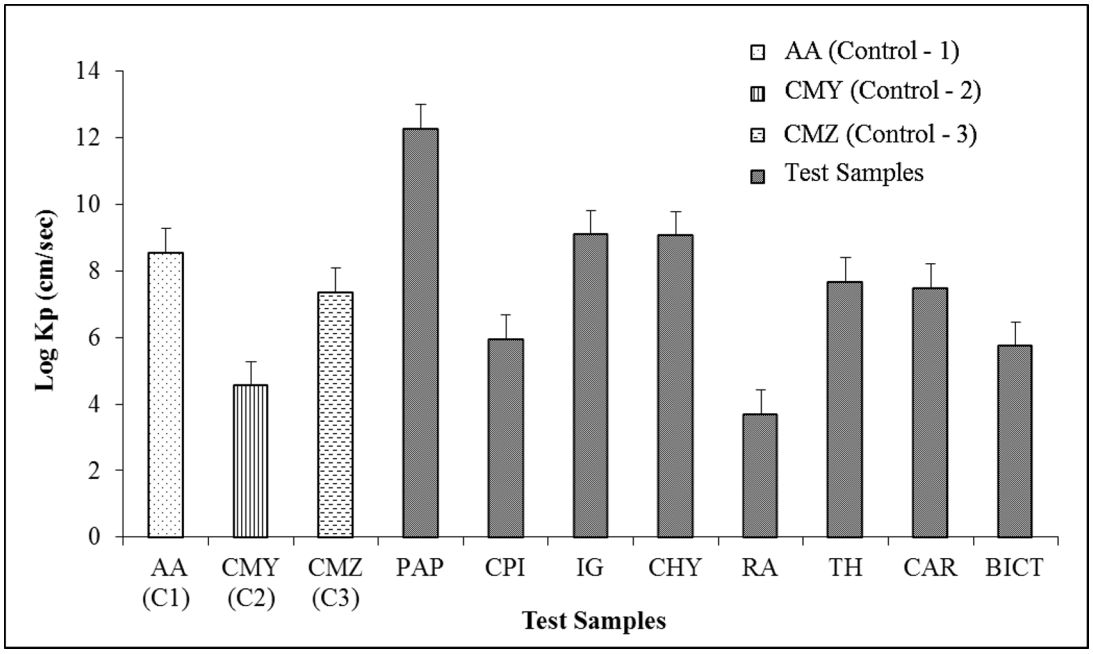

Figure 6. Comparative skin-permeation rates of the screened phytoconstituents.

3.4 Antimicrobial Activity

The antimicrobial assay indicated that *C. papaya* extract exhibited greater overall antifungal activity than antibacterial activity (Figure 7). The largest zone of inhibition was observed against *R. oryzae*, while the smallest was observed against *M. luteus*. These findings indicate that the ethanolic leaf extract possesses measurable broad-spectrum activity, with comparatively stronger performance against the fungal strains tested.

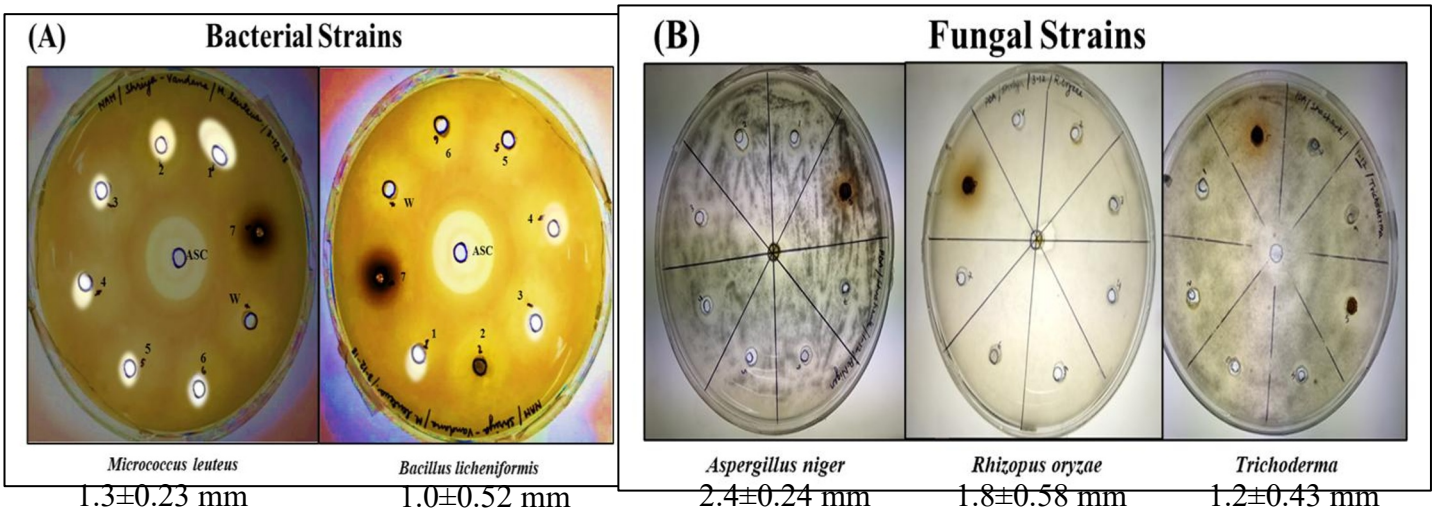

Figure 7. Antibacterial and antifungal activity of Carica papaya extract against the selected test organisms.

3.5 Antioxidant Activity (DPPH Assay)

The DPPH assay showed a clear concentration-dependent antioxidant response (Figure 8). Percentage inhibition increased from 86.59% at 200 µg/mL to 98.31% at 1 mg/mL, indicating strong radical-scavenging capacity. This antioxidant effect may support the broader therapeutic relevance of the extract in dermal applications, where oxidative stress often contributes to tissue damage and delayed healing.

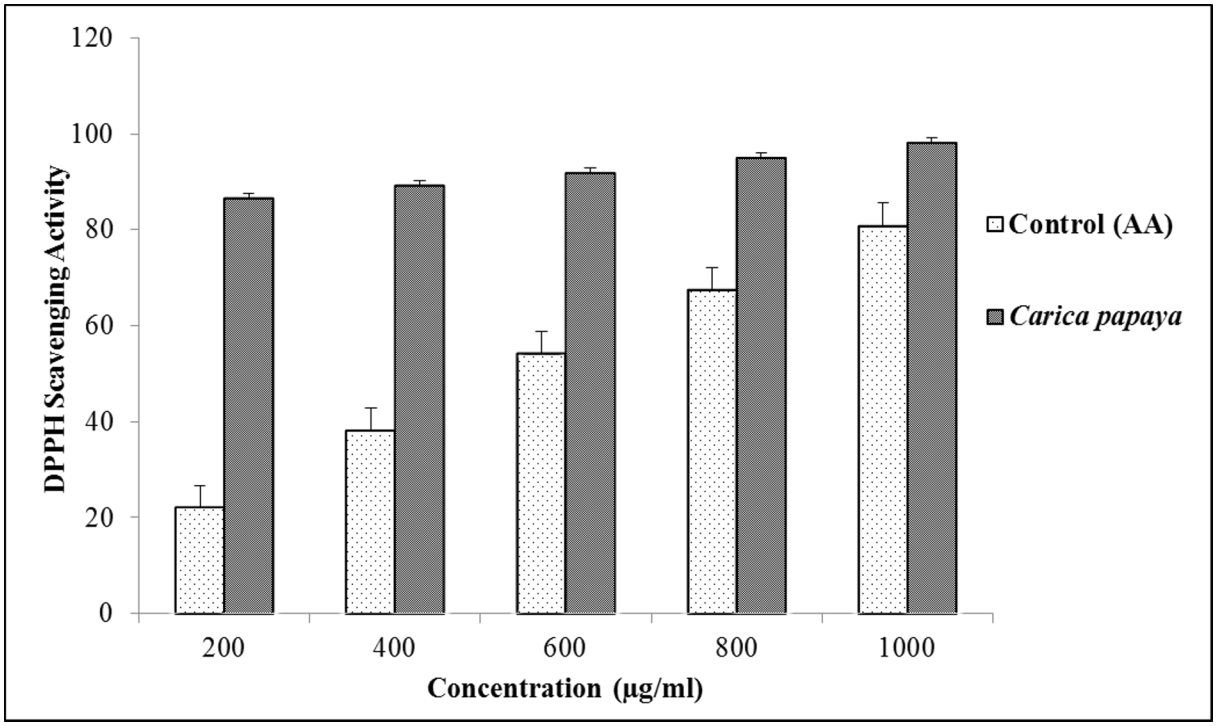

Figure 8. Antioxidant evaluation of Carica papaya leaf extract by the DPPH assay.

4. Discussion

The present study combined phytochemical screening, antimicrobial testing, antioxidant evaluation, docking analysis, toxicity prediction, and skin-permeation assessment to examine the therapeutic potential of C. papaya leaf extract in the context of dermal infections. By integrating experimental observations with preliminary molecular interpretation, the study provides a more comprehensive evaluation than any single approach alone.

Among the screened phytoconstituents, cysteine protease inhibitor consistently showed the strongest predicted binding to the selected bacterial and fungal target proteins, while chymotrypsin also showed favourable docking scores. It should be noted that these targets are proteins associated with β-glucan, mannan, lipoteichoic acid, and N-acetylglucosamine-related pathways, and not the polysaccharide components themselves. This distinction is important for the biological interpretation of the docking results and avoids the misconception that docking was performed directly against glucan or mannan polymers.

The in vitro data showed stronger antifungal activity than antibacterial activity, particularly against R. oryzae. While docking and agar well diffusion results cannot be directly equated, the computational observations offer plausible molecular support suggesting that some papaya-derived compounds may interact with proteins involved in microbial cell-wall integrity. However, the experimental assay was conducted on crude extract, whereas docking examined individual compounds. The computational findings should therefore be viewed as supportive rather than definitive explanations of the observed antimicrobial effect.

Toxicological prediction indicated that most evaluated phytoconstituents fall within acceptable exploratory toxicity ranges, although carpine showed a comparatively less favourable profile than the other screened compounds. The skin-permeation analysis further indicated that several phytoconstituents possess physicochemical profiles compatible with topical exposure. Taken together, these observations support the candidacy of C. papaya for further topical formulation studies.

The strong DPPH radical-scavenging activity further supports the functional relevance of the extract, particularly given that oxidative stress commonly accompanies infection-associated inflammation and delayed wound repair. The combined antimicrobial and antioxidant activities may therefore be advantageous for dermal applications, although detailed compound isolation, formulation studies, and in vivo validation are still needed.

Overall, the data position C. papaya leaf extract as a promising natural source of bioactive molecules with antimicrobial potential. While the present findings are encouraging, future work should include compound quantification, target-specific validation, and expanded formulation studies to build translational relevance.
Further in vitro and in vivo studies are therefore required to confirm the contribution of individual phytoconstituents, validate the predicted molecular interactions, and assess dermatological safety in formulated preparations.

5. Conclusion

Carica papaya leaf extract demonstrated notable antioxidant and antimicrobial activity and showed stronger antifungal than antibacterial effects under the conditions tested. The accompanying docking analysis provided preliminary molecular support by identifying favourable interactions between selected phytoconstituents and proteins associated with microbial cell-wall pathways. Together with the toxicity and skin-permeation predictions, these findings support the potential of C. papaya as a promising source of bioactive constituents for future topical antimicrobial development.

Acknowledgements

The authors acknowledge Jaypee Institute of Information Technology, Noida, for providing the entire infrastructure to complete this project.

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Abbreviations: AA, ascorbic acid; BICT, benzyl isothiocyanate; CAR, carpine; CHY, chymotrypsin; CMY, clindamycin; CMZ, clotrimazole; CPI, cysteine protease inhibitor; IG, indolyglucosinolate; NAG, N-acetylglucosamine-associated target; PAP, papain; RA, retinoic acid; TH, thiamine.