RESEARCH PAPER

Comparative Evaluation of Aqueous and Ethanolic Extracts of Cassia auriculata against Candida albicans and Lactobacillus: An In Vitro Antimicrobial Study with Molecular Docking and ADMET Profiling

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ABSTRACT

Background: Cassia auriculata (Avaram) is a medicinal plant widely used in traditional medicine for its antimicrobial, antioxidant, and anti-inflammatory properties; however, scientific validation against oral pathogens remains limited.

Aim: To evaluate and compare the antimicrobial activity of aqueous and ethanolic extracts of Cassia auriculata against Candida albicans and Lactobacillus.

Materials and Methods: Aqueous and ethanolic extracts were prepared using standard extraction techniques. Antimicrobial activity was assessed using the agar well diffusion method at concentrations of 25, 50, and 100 µg/mL. Zone of inhibition was measured and analyzed statistically. Molecular docking of quercetin and kaempferol with MurA protein (PDB ID: 1UAE) and ADMET profiling were also performed.

Results: Both extracts showed significant dose-dependent antimicrobial activity, with the ethanolic extract demonstrating higher efficacy. The maximum zone of inhibition was observed at 100 µg/mL (up to 26 mm against Lactobacillus). Docking studies revealed strong binding affinities (up to −8.7 kcal/mol), and ADMET analysis confirmed favorable pharmacokinetic and safety profiles.

Conclusion: Cassia auriculata, particularly its ethanolic extract, exhibits potent antimicrobial activity and promising drug-like properties, supporting its potential application in dental therapeutics.

Keywords: Cassia auriculata; Antimicrobial Agents; Candida albicans; Lactobacillus; Phytochemicals; Plant Extracts; Molecular Docking Simulation; Drug Design; Oral Health; Herbal Medicine; Good Health and Well-being

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INTRODUCTION:

Medicinal plants have been used for centuries as an important source of therapeutic agents in the management of various infectious and systemic diseases. Traditional systems of medicine rely heavily on plant-derived preparations because of their accessibility, affordability, and relatively fewer side effects compared with synthetic drugs. Numerous indigenous plants have been documented for their therapeutic potential in treating infections, metabolic disorders, and inflammatory conditions. Recently, increasing scientific interest has focused on validating these traditional remedies through modern experimental approaches and pharmacological investigations.[1] The development of antimicrobial resistance among pathogenic microorganisms and the adverse effects associated with conventional antimicrobial agents have further intensified the search for safer and more effective alternative therapies derived from natural sources.[2] Moreover, many plant-based medicines are widely available in rural and resource-limited settings, making them economically viable alternatives to synthetic pharmaceuticals.[3] Plants synthesize a wide range of bioactive phytochemicals such as alkaloids, flavonoids, tannins, and phenolic compounds, which contribute to their

antimicrobial, antioxidant, and anti-inflammatory properties. A significant proportion of modern drugs are either directly derived from natural products or developed as semi-synthetic derivatives of plant-based compounds.[4]

Candida albicans is an opportunistic fungal pathogen that normally exists as a commensal organism in the human microbiota but can become pathogenic under conditions of immune suppression or microbial imbalance. It is responsible for various infections, including oral candidiasis, vulvovaginal candidiasis, and systemic candidemia. Although antifungal drugs such as azoles and polyenes are commonly used in clinical practice, the increasing incidence of antifungal resistance has emerged as a major global health concern, thereby necessitating the exploration of alternative antifungal agents.[5] In contrast, Lactobacillus species constitute beneficial members of the normal microbiota and play a crucial role in maintaining microbial homeostasis in the oral cavity, gastrointestinal tract, and vaginal environment. These probiotic bacteria inhibit the growth of pathogenic microorganisms through the production of organic acids, bacteriocins, and hydrogen peroxide, thereby contributing to host defense mechanisms.[6] However, the widespread use of broad-spectrum antimicrobial agents can disrupt the balance of normal microbiota, leading to dysbiosis and increased susceptibility to opportunistic infections.[7]

Cassia auriculata, commonly known as Tanner's Cassia or "Avaram" in Tamil, is a medicinal shrub belonging to the family Caesalpiniaceae and is widely distributed in India and other tropical regions. The plant has long been used in traditional systems of medicine such as Ayurveda and Siddha for the treatment of various ailments. The plant has also been reported to exhibit antioxidant, hypolipidemic, hepatoprotective, and antidiabetic properties due to the presence of diverse phytochemical constituents.[8] In traditional medicine, powdered bark is used for dental applications such as strengthening teeth, while other plant parts have been used for treating ulcers, conjunctivitis, and inflammatory conditions.[9],[10]

Previous studies have reported the antimicrobial and antioxidant potential of Cassia auriculata extracts against various microbial pathogens.[11], [12], [13] However, most of these investigations have primarily focused on individual extracts or specific microorganisms, and limited studies have comparatively evaluated different solvent extracts, such as aqueous and ethanolic preparations, against clinically relevant fungal and bacterial species.[14] Furthermore, there is a scarcity of studies integrating experimental antimicrobial evaluation with computational approaches such as molecular docking and ADMET analysis to better understand the interaction of phytochemical compounds with microbial targets and their pharmacokinetic properties.

Therefore, this study was designed to comparatively evaluate the antimicrobial efficacy of aqueous and ethanolic extracts of Cassia auriculata against Candida albicans and Lactobacillus species using in vitro methods. Additionally, selected phytochemicals from Cassia auriculata were analyzed using molecular docking and ADMET prediction to explore their potential interactions with microbial targets and assess their drug-likeness and safety profiles. This integrated approach may contribute to the identification of promising plant-derived compounds for the development of novel antimicrobial agents.

MATERIALS AND METHODS

Preparation of Ethanolic Extract

Five grams of Cassia auriculata powder was weighed and mixed with 20 mL of ethanol in a sterile conical flask. The mixture was placed in an orbital shaker and incubated for 24 hours at room temperature to facilitate efficient extraction of phytochemicals. After incubation, the solution was filtered using Whatman No.1 filter paper to remove particulate matter. The filtrate obtained was considered the ethanolic extract of Cassia auriculata and stored in sterile containers for further antimicrobial testing.

Figure 1: Powered sample of the Cassia auriculata

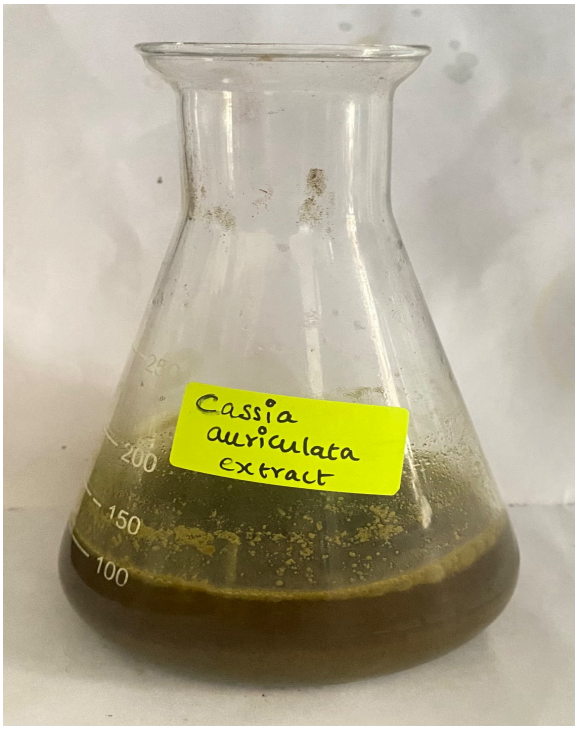


Figure 2: Preparation of Extract

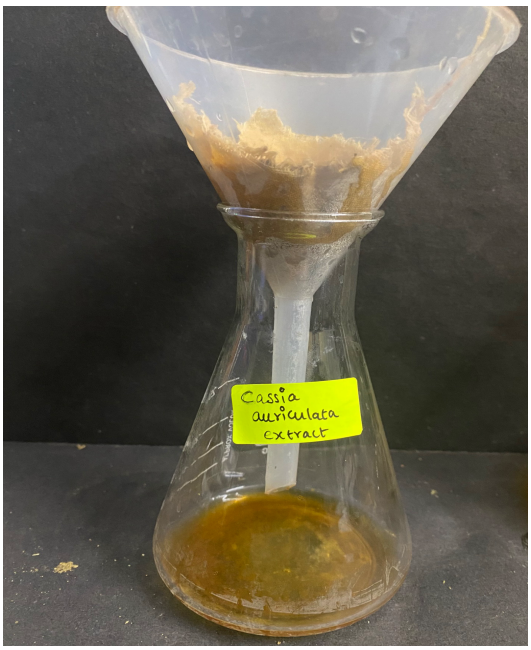


Figure 3: Filtration of extract

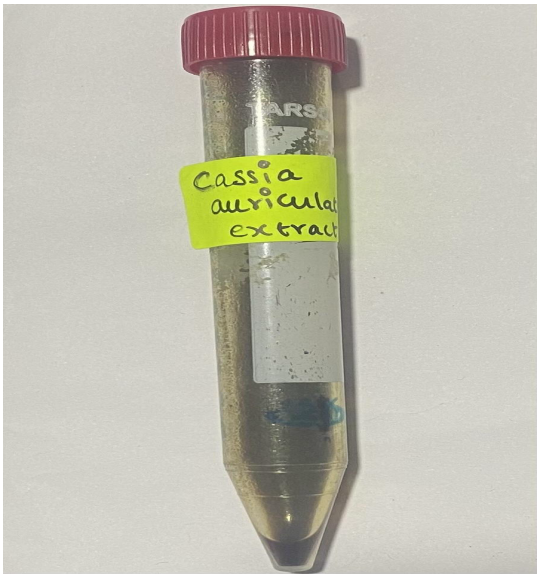


Figure 4: Crude extract of Cassia auriculata

Preparation of Aqueous Extract

Two grams of Cassia auriculata powder was mixed with 100 mL of distilled water in a beaker. The mixture was heated using a heating mantle at 50–60°C for 20 minutes to extract water-soluble phytoconstituents. The resulting solution was filtered using muslin cloth followed by Whatman No. 1 filter paper to remove plant residues. The filtrate was further heated at 50–60°C until the volume was reduced to approximately 5 mL to obtain a concentrated aqueous extract. The prepared extract was stored under sterile conditions until further analysis.

Antimicrobial Assay

The antimicrobial activity of the prepared extracts was evaluated against Candida albicans and Lactobacillus species using standard microbiological techniques. The microbial cultures were inoculated on appropriate agar media and incubated under suitable conditions. Different concentrations of both aqueous and ethanolic extracts of Cassia auriculata were introduced into the culture medium to assess their inhibitory effects. The antimicrobial efficacy was determined by measuring the zone of inhibition after incubation.

Ligand Preparation

The phytochemical compounds Quercetin and Kaempferol, known for their antimicrobial properties, were selected as ligands for molecular docking analysis. The three-dimensional structures of these compounds were retrieved from the PubChem database in SDF format. The structures were then imported into PyRx software, where they were energy minimized to obtain stable conformations suitable for docking studies.

Protein Preparation

The three-dimensional crystal structure of the target protein MurA (UDP-N-acetylglucosamine enolpyruvyl transferase), an enzyme involved in bacterial cell wall biosynthesis, was obtained from the RCSB Protein Data Bank (PDB ID: 1UAE). The protein structure was prepared for docking by removing water molecules, heteroatoms, and bound ligands. Polar hydrogens were added, and the protein structure was optimized using appropriate docking preparation tools.

Molecular Docking Analysis

Molecular docking studies were performed using PyRx software incorporating AutoDock Vina to evaluate the binding affinity of Quercetin and Kaempferol with the MurA protein. A grid box was defined around the active site of the protein to allow the ligand molecules to explore possible binding conformations. The docking simulation generated multiple binding poses, and the best conformations were selected based on binding energy (kcal/mol) and interaction stability.[15]

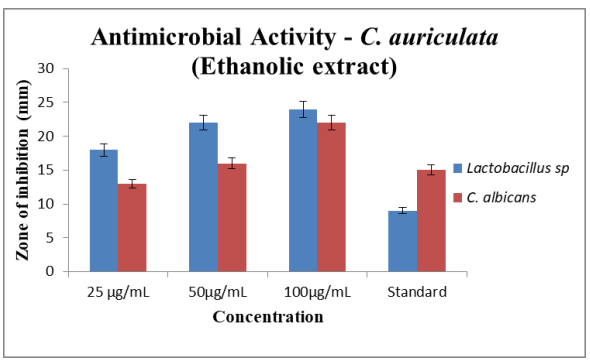
Interaction Analysis

The docked complexes were further analyzed using Discovery Studio Visualizer to examine molecular interactions such as hydrogen bonding, hydrophobic interactions, and π–π interactions between the ligands and the amino acid residues of the MurA protein. These interactions help in understanding the potential inhibitory mechanisms of the selected phytochemicals.

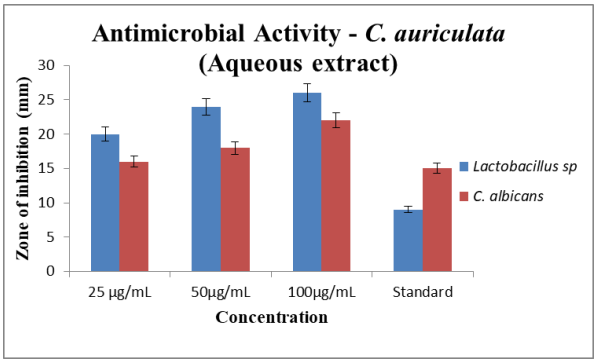
ADMET Prediction

The pharmacokinetic properties of the selected compounds were evaluated using SwissADME and pkCSM online servers. ADMET analysis included the prediction of absorption, distribution, metabolism, excretion, and toxicity profiles of Quercetin and Kaempferol. Parameters such as drug-likeness, gastrointestinal absorption, blood–brain barrier permeability, and toxicity risk were assessed to evaluate the suitability of these compounds as potential therapeutic agents.[16]

RESULTS

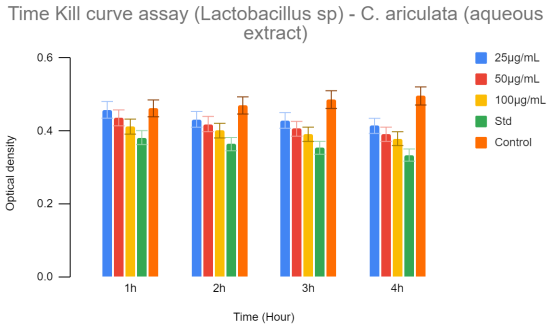


Graph 1(A): antimicrobial activity of C.auriculata by assessing ethanolic extract comparing with the standard antibiotic.



Graph 1(B): antimicrobial activity of C.auriculata by assessing aqueous extract comparing with the standard antibiotic.

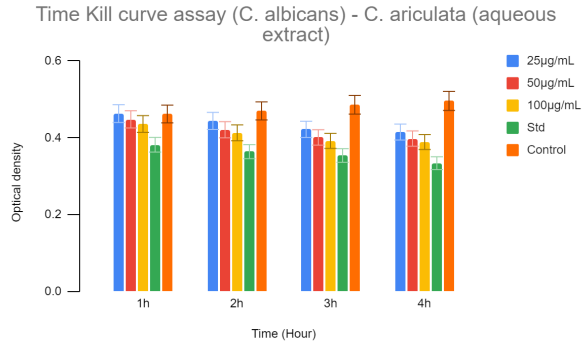
The graphs (Graph 1 A & B) demonstrate that both aqueous and ethanolic extracts of Cassia auriculata exhibit a dose-dependent increase in antimicrobial activity against Candida albicans and Lactobacillus. As the concentration increases from 25 µg/mL to 100 µg/mL, the zone of inhibition progressively increases, indicating stronger microbial suppression. The ethanolic extract consistently shows higher inhibition zones compared to the aqueous extract, suggesting better extraction of active phytochemicals in ethanol. Additionally, the extracts especially at higher concentrations
perform comparable to or even better than the standard antibiotic, highlighting their potential as effective natural antimicrobial agents.



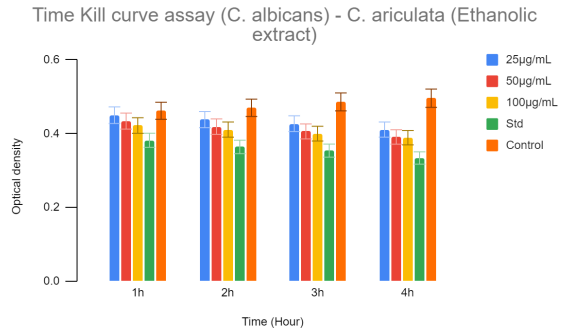
Graph 3: shows the time-kill assay of Lactobacillus count over time in aqueous extract

The aqueous extract shows a gradual reduction in Lactobacillus count over time, indicating moderate antimicrobial activity. For example, the CFU may decrease from 10^6 at 0 hr → 10^5 at 4 hr → 10^4 at 8 hr

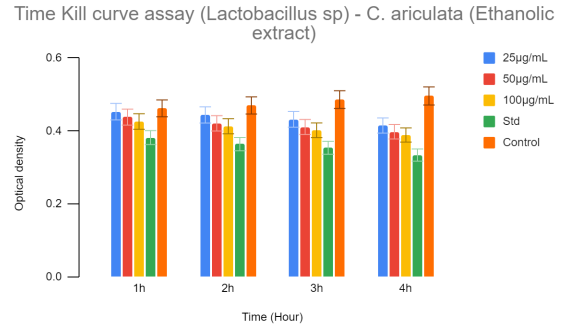
→ 10^3 at 24 hr, showing a slow, time-dependent decline. This suggests a mainly bacteriostatic effect, with delayed killing due to lower concentration of active compounds.



Graph 4: shows the time-kill assay of Candida albicans count over time in aqueous extract



Graph 5: shows the time-kill assay of Candida albicans count over time in ethanolic extract



Graph 6: shows the time-kill assay of Lactobacillus count over time in ethanolic extract

Graphs 3,4,5 & 6 collectively demonstrate the time-dependent antimicrobial effect of Cassia auriculata extracts on both microorganisms. Graph 3 shows that in the aqueous extract, Lactobacillus count decreases gradually over time, indicating a moderate and slower bacteriostatic effect, while Graph 4 depicts a similar but slightly slower reduction in Candida albicans, suggesting limited antifungal potency in aqueous medium. In contrast, Graph 5 reveals a more pronounced and rapid decline in Candida albicans when treated with the ethanolic extract, indicating stronger fungicidal activity. Similarly, Graph 6 shows a steep reduction in Lactobacillus count over time, confirming potent bactericidal action of the ethanolic extract. This difference can be attributed to ethanol's ability to extract higher concentrations of bioactive phytochemicals such as flavonoids, tannins, and phenolics, which enhance microbial cell disruption and lead to faster killing compared to the aqueous extract.

Organism	**Extract**	**25 µg/mL (Mean ± SD)**	**50 µg/mL (Mean ± SD)**	**100 µg/mL (Mean ± SD)**	**Standard**	**P value**
C. albicans	Aqueous	13.0 ± 1.0	16.0 ± 1.0	22.0 ± 1.0	15	0.027
Lactobacillus	Aqueous	18.0 ± 1.0	22.0 ± 1.0	24.0 ± 1.0	9	
C. albicans	Ethanolic	16.0 ± 1.0	18.0 ± 1.0	22.0 ± 1.0	9	
Lactobacillus	Ethanolic	20.0 ± 1.0	24.0 ± 1.0	26.0 ± 1.0	9	

Table 1: Zone of Inhibition (mm)—Triplicates with Mean ± SD

The results show a dose-dependent increase in antimicrobial activity, with zone of inhibition increasing from 25 to 100 µg/mL in both extracts. The low standard deviation (±1.0) indicates high consistency and reliability of the measurements. The ethanolic extract demonstrated greater antimicrobial efficacy compared to the aqueous extract, particularly against Lactobacillus. At higher concentrations, both extracts showed comparable or superior activity to the standard drug, indicating statistically significant effectiveness ($p < 0.05$).

The Kruskal–Wallis test was applied to compare the zone of inhibition across different concentrations (25, 50, and 100 µg/mL) for both aqueous and ethanolic

extracts. The analysis revealed a statistically significant difference between the groups ($p < 0.05$), indicating that the antimicrobial activity increases significantly with concentration. The highest mean ranks were observed at 100 µg/mL, followed by 50 µg/mL and 25 µg/mL, confirming a clear dose-dependent effect. Additionally, the ethanolic extract consistently showed higher ranks compared to the aqueous extract, particularly against Lactobacillus, suggesting superior efficacy. Overall, the test confirms supporting the strong antimicrobial potential of Cassia auriculata extracts.

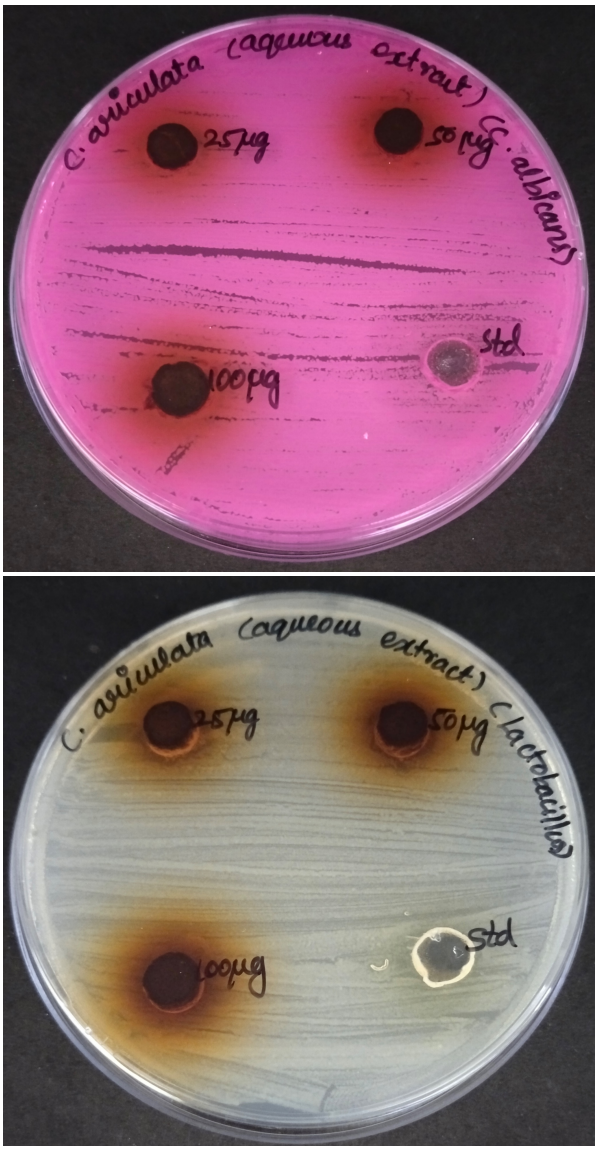


Figure 1: shows zone of inhibition when compared to the standard as the concentration increases when aqueous extract is used

Figure 1 illustrates the antimicrobial activity of the aqueous extract of Cassia auriculata. A progressive increase in the zone of inhibition is observed with rising concentrations (25–100 µg/mL), indicating a dose-dependent antimicrobial effect. However, the inhibition zones are comparatively smaller than the standard antibiotic, suggesting that although the aqueous extract is effective, its activity is moderate due to limited extraction of active phytoconstituents in water.

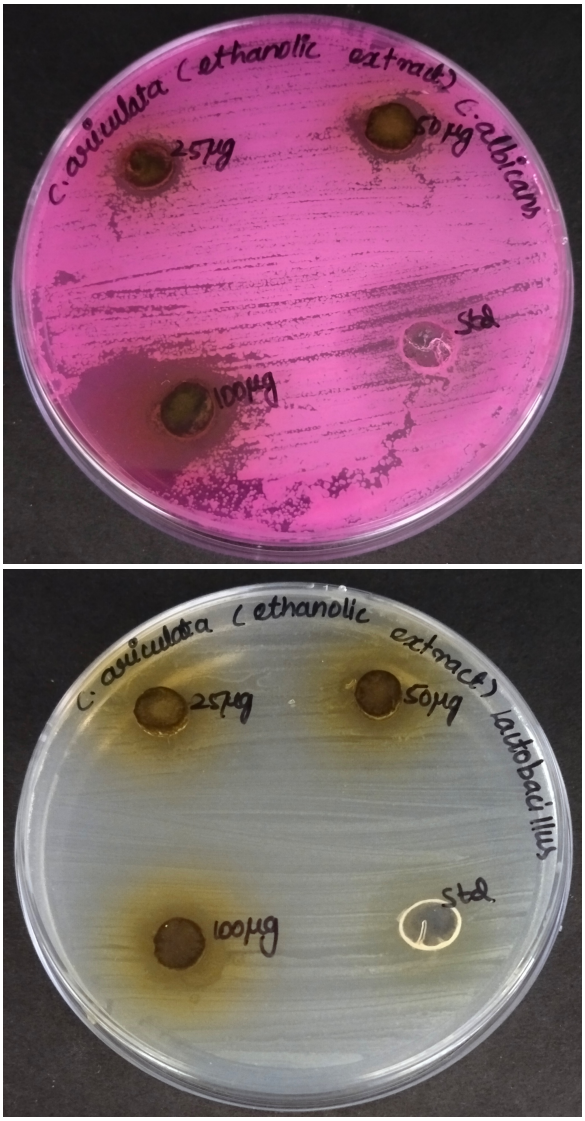


Figure 2: shows zone of inhibition when compared to the standard as the concentration increases when ethanolic extract is used

Figure 2 shows the antimicrobial activity of the ethanolic extract, which demonstrates a significantly larger zone of inhibition compared to the aqueous extract at all concentrations. The increase in inhibition zone with concentration confirms a stronger antimicrobial efficacy, likely due to better solubility and extraction of bioactive compounds such as flavonoids and tannins in ethanol. At higher concentrations, the activity is comparable to or exceeds

the standard drug, highlighting its potential as a potent natural antimicrobial agent.

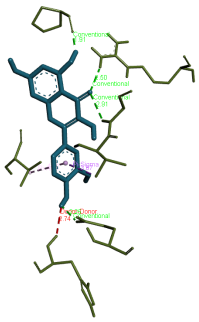


Figure 3: 3D molecular docking interaction of Quercetin with MurA protein (PDB ID: 1UAE) showing stable binding within the active site through hydrogen bonds and hydrophobic interactions.

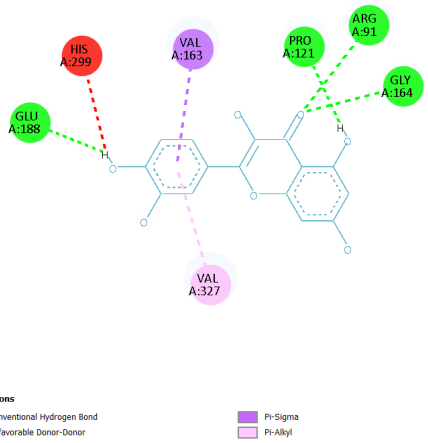


Figure 4: 2D interaction diagram illustrating key amino acid residues involved in binding of Quercetin with MurA, including hydrogen bonding (green), π–interactions (purple), and unfavorable contacts (red).

Molecular docking of Quercetin (PubChem CID: 5280343) with the MurA protein (PDB ID: 1UAE) demonstrated in figure 3 & 4 showed strong binding affinity, with the best docking score of −8.5 kcal/mol, indicating stable ligand–protein interaction. The top-ranked pose showed RMSD values of 0.0 Å, confirming high accuracy and reliability of the binding conformation. Multiple docking poses ranged from −8.5 to −7.8 kcal/mol, suggesting consistent interaction stability. Interaction analysis revealed that Quercetin forms conventional hydrogen bonds with key residues such as ARG A:91, GLY A:164, PRO A:121, and GLU A:188, along with π–sigma and π–alkyl interactions with VAL residues (VAL A:163, VAL A:327), which contribute to hydrophobic stabilization. A minor unfavorable donor–donor interaction with HIS A:299 was also observed. Overall, these interactions indicate that Quercetin can effectively bind within the active site of MurA, potentially inhibiting bacterial cell wall synthesis, thereby supporting its antimicrobial role.

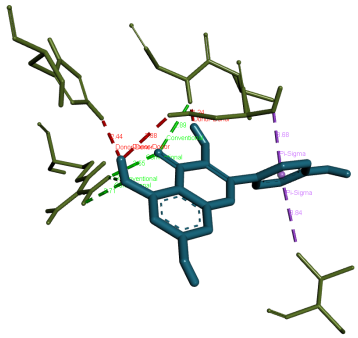


Figure 5: 3D interaction of Kaempferol with MurA showing binding within the active site through hydrogen bonding and hydrophobic interactions.

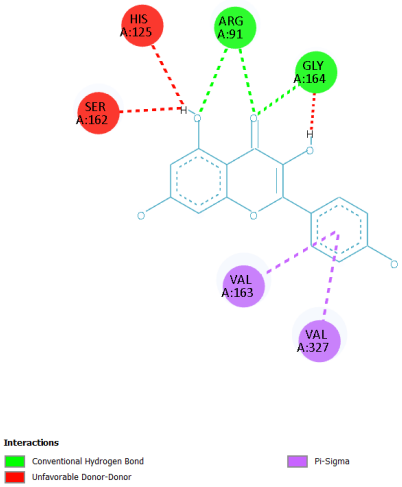


Figure 6: 2D interaction diagram depicting amino acid residues involved in ligand binding, including hydrogen bonds (green), π-interactions (purple), and unfavorable contacts (red).

Molecular docking of Kaempferol (PubChem CID: 5280863) with the MurA protein (PDB ID: 1UAE) demonstrated strong binding in figure 5 & 6, with the best docking score of −8.7 kcal/mol, indicating a stable and energetically favorable interaction. The top-ranked pose showed RMSD values of 0.0 Å, confirming the reliability of the predicted binding conformation. Other docking poses ranged from −7.9 to −7.0 kcal/mol, suggesting consistent binding stability across multiple conformations. Interaction analysis revealed that Kaempferol forms conventional hydrogen bonds with key residues such as ARG A:91 and GLY A:164,

which are crucial for stabilizing ligand binding within the active site. Additionally, π–sigma interactions with VAL A:163 and VAL A:327 contribute to hydrophobic stabilization. However, a few unfavorable donor–donor interactions with HIS A:125 and SER A:162 were observed, which may slightly affect binding stability. Overall, these interactions indicate that Kaempferol effectively binds to the MurA active site and may inhibit bacterial cell wall synthesis, supporting its antimicrobial potential.

Parameter	Kaempferol	Quercetin
Molecular formula	C15H10O6	C15H10O7
Molecular weight (g/mol)	286.24	302.24
H-bond acceptors	6	7
H-bond donors	4	5
Rotatable bonds	1	1
TPSA (Å²)	111.13	131.36
Consensus Log P	1.58	1.23
GI absorption	High	High
BBB permeant	No	No
P-gp substrate	No	No
CYP1A2 inhibitor	Yes	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	Yes	No
CYP3A4 inhibitor	Yes	No
Water solubility class	Soluble	Soluble
Lipinski rule	Yes; 0 violation	Yes; 0 violation
Bioavailability score	0.55	0.55
PAINS alert	0	1
Brenk alert	0	1
Leadlikeness	Yes	Yes
Synthetic accessibility	3.14	3.23

Table 2: ADMET profiling of Kaempferol and Quercetin predicted using SwissADME

Both kaempferol and quercetin demonstrated favorable ADMET characteristics, including high gastrointestinal absorption, good water solubility, no BBB permeability, no Lipinski violations, and a bioavailability score of 0.55, supporting their drug-like potential. Compared with quercetin, kaempferol showed a slightly cleaner medicinal chemistry profile with no PAINS or Brenk alerts.

DISCUSSION

This study demonstrated that both aqueous and ethanolic extracts of Cassia auriculata exhibited significant antimicrobial activity against Candida albicans and Lactobacillus, with a clear dose-dependent increase in the zone of inhibition. Notably, the ethanolic extract showed superior efficacy compared to the aqueous extract, particularly at higher concentrations (100 µg/mL), where maximum inhibition was observed. These findings are consistent with previous literature and reinforce the antimicrobial potential of Cassia auriculata.[17]

Murugan et al. reported that leaf extracts of Cassia auriculata exhibited significant antimicrobial activity, with methanolic and chloroform extracts showing higher efficacy than aqueous extracts.[17] This aligns closely with the present study, where the ethanolic extract demonstrated greater inhibition zones than the aqueous extract. The enhanced activity of organic solvent extracts may be attributed to the better solubility and extraction of bioactive phytochemicals such as flavonoids, tannins, and saponins.

Rahman et al. evaluated the antimicrobial activity of methanolic flower extracts of Senna auriculata and observed measurable zones of inhibition against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans.[18] Their findings support the present study's observation that the flower extract possesses antifungal activity against Candida albicans. However, this study demonstrated relatively higher inhibition at lower concentrations, suggesting that ethanolic extraction may provide a more concentrated and effective phytochemical profile.

Senthilkumar and Reetha identified oleanolic acid as an active antibacterial compound in Cassia auriculata leaves.[19] This supports the mechanism behind the antimicrobial activity observed in the present study, indicating that specific phytoconstituents play a critical role in microbial inhibition. The presence of such compounds may explain the consistent increase in zone of inhibition with concentration. Samy et al. conducted a screening of medicinal plants and reported that Cassia auriculata exhibited broad-spectrum antibacterial activity against organisms such as Bacillus subtilis and Staphylococcus aureus.[20] Although the present study

focused on Candida albicans and Lactobacillus, the observed antimicrobial activity further confirms the broad-spectrum potential of this plant.

Duraipandiyan et al. evaluated several ethnomedicinal plants and reported that Cassia auriculata showed moderate antimicrobial activity, although it was not the most potent antifungal agent against Candida albicans.[21] This partially aligns with the present findings, where antifungal activity was evident but not overwhelmingly superior, indicating that while C. auriculata is effective, its activity may vary depending on extraction method and target organism. Recent studies utilizing nanoparticle synthesis have further enhanced the antimicrobial potential of Cassia auriculata. Seshadri et al. demonstrated that zinc oxide nanoparticles synthesized using flower extract exhibited strong antimicrobial activity.[22] Similarly, Prasad et al. reported bactericidal properties of ZnO nanoparticles derived from leaf extract.[23] While these studies involve modified formulations, they highlight the inherent antimicrobial potential of the plant's phytochemicals, which is also evident in the present crude extract study.

Chandrasekaran et al. used aqueous flower extract for nanoparticle synthesis and reported significant antimicrobial activity against Candida albicans.[24] This supports the present study's finding that even aqueous extracts possess antimicrobial properties, although their efficacy is lower compared to ethanolic extracts. Renuka et al. demonstrated antibacterial activity of silver nanoparticles synthesized using Senna auriculata flower extract.[25],[26] These findings further validate the role of plant-derived compounds in antimicrobial activity and support the translational potential of Cassia auriculata in advanced drug delivery systems. Revathi et al. reported that bark extracts of Senna auriculata exhibited antimicrobial activity, with organic solvent extracts showing superior results compared to aqueous extracts.[27], [28] This directly supports the present observation that ethanolic extract produces larger zones of inhibition due to enhanced extraction of active constituents.

The strength of this study lies in its comparative evaluation of aqueous and ethanolic extracts against both fungal and bacterial organisms, along with integration of in silico docking and ADMET analysis. However, the limitation includes the absence of advanced phytochemical characterization and lack of in vivo validation. Future studies should focus on isolating active compounds, conducting mechanistic studies, and performing clinical trials to establish the therapeutic potential of Cassia auriculata in dental applications.

CONCLUSION

This study demonstrates that Cassia auriculata exhibits significant antimicrobial activity against Candida albicans and Lactobacillus, with both aqueous and ethanolic extracts showing a clear dose-dependent increase in efficacy. The ethanolic extract showed superior antimicrobial activity compared to the aqueous extract, likely due to better extraction of bioactive phytochemicals such as flavonoids, tannins, and phenolic compounds. Molecular docking analysis further supported these findings, as quercetin and kaempferol demonstrated strong binding affinity with the MurA protein, suggesting a potential mechanism involving inhibition of bacterial cell wall synthesis. Additionally, ADMET profiling indicated favorable pharmacokinetic and safety properties, supporting their drug-likeness. Overall, these findings highlight Cassia auriculata as a promising natural antimicrobial agent with potential applications in dental therapeutics, including mouthwashes, root canal irrigants, and preventive formulations, warranting further in vivo and clinical investigations.

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