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RESEARCH ARTICLE

Inhibitory Activity of Gallic Acid and Caffeic Acid from Honey on Plasma Membrane Protein RCh1P Of Candida Albicans

Arun Ganesh M K[1], Sathish Sankar[2]*

[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Velappanchavadi, Chennai - 600 077. Tamil Nadu, India. Phone: +91-9941011800 Email: 152001083.sdc@saveetha.com

[2]Sathish Sankar, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Poonamallee High Road, Chennai – 600 077, Tamil Nadu, India. Phone: +91-9600690306 Email: sathish3107@gmail.com ORCID: 0000-0002-9389-5231 Conflict of Interest: None to declare

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*Corresponding Author
Sathish Sankar

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Abstract: Candidiasis incidence has increased, mostly due to the use of plastic permanent catheters, antibiotics and immunosuppressive drugs. These Candida-derived infections may occur in the skin, mucous membranes and viscera, with the main etiological agent being Candida albicans. Gallic acid (GA), is a polyphenol acid compound and it is ubiquitous in fruits, vegetables, and herbal medicines, such as grapes, gallnuts, pomegranates, and tea leaves. The RCh1P protein was modelled using Swiss-Model online server program. Phytochemicals identified from honey namely, caffeic acid and gallic acid, were selected for the study. Molecular docking was carried out using Hex Protein Docking server. The structures were viewed, and analysed for polar hydrogen atoms, and identification of binding residues was carried out using PyMOL program ver. 2.4.0. Interaction of the drug including gene expression, possible adverse effects and side effects were predicted using pass online web server. The physiochemical properties, lipophilicity, water solubility, pharmacokinetics and drug-likeliness were assessed using SwissADME online sever program. The highly negative E-total scores as a result of molecular docking of Gallic acid and caffeic acid with RCh1P plasma membrane protein of Candida species, demonstrates the inhibitory activity of the phytochemicals on the growth of the fungus. This study indicates that the effect of Gallic acid and Caffeic acid on plasma membrane protein RCh1P is inhibitory.

Keywords: Caffeic acid, Candidiasis, Gallic acid, Honey, Molecular docking, Phytochemicals.

INTRODUCTION

The use of plastic permanent catheters, antibiotics, and immunosuppressive medications are the main causes of the rise in candidiasis incidence during the 1970s(Tayel et al., 2010). *Candida albicans* is the primary etiological agent of these Candida-derived infections, which can affect the skin, mucous membranes (including the mouth and vagina), and viscera(Sgherri et al., 2014). With overall fatality rates ranging from 29 to 76 percent, *C. albicans*, one of the many human fungal pathogens, is responsible for the majority of systemic infections in immunocompromised individuals(Wisplinghoff et al., 2014). Due to its resistance to the majority of antimicrobial substances, including amphotericin-B, the standard antifungal agent for treating systemic mycoses, this opportunistic fungus presents serious issues(Lortholary et al., 2014). These antifungal medications are still widely regarded as the best treatment for *C. albicans*, however there have been an increasing number of reports of their ineffectiveness due to resistance, notably to fluconazole(Bassetti et al., 2013). Due to this issue, researchers are looking for alternate medications and chemical substances to utilize in the management and treatment of *C. albicans* infections.

Because it is found in a wide variety of fruits, vegetables, and herbal medicines such grapes, gallnuts, pomegranates, and tea leaves, gallic acid (GA), also known as 3,4,5-trihydroxybenzoic acid, has steadily gained a lot of interest(Ferruzzi et al., 2009). Famous Swedish chemist Carl Wilhelm Scheele was the first to recognise and isolate GA and pyrogallic acid from plants in 1786(Wang & Li, 2017). There are several uses for gallic acid and its derivatives. Pharmaceuticals, paints, colour developers, and inks all use gallic acid as a raw material(J. Zhang et al., 2018). Propyl gallate, octyl gallate, lauryl gallate, and dodecyl gallate are examples of gallic acid esters that are frequently employed as food additives and antioxidants in cosmetics(Singh et al., 2014). Propyl gallate is a common antioxidant used in the pharmaceutical business and is effective in reducing rancidity and deterioration of fats and oils(Y. Zhang et al., 2026).

The primary hydroxycinnamic acid present in the diets of people is caffeic acid (CA), a polyphenol formed by the secondary metabolism of vegetables such as olives, coffee beans, fruits, potatoes, carrots, and propolis(R. P. Verma & Hansch, 2004). This phenolic substance can be found in the simple form (monomers) as organic acid esters, sugar esters, amides, and glycosides or in more complicated forms like dimers, trimers, and derivatives of flavonoids(Huang et al., 2013). It can also be found attached to proteins and other polymers in the vegetable's cell wall(Genaro-Mattos et al., 2015). Because CA inhibits the growth of bacteria, fungi, and insects and promotes the protection of plant leaves against ultraviolet radiation, it contributes to the defensive

system of plants against predators, pests, and illnesses (UV-B)(Lin & Yan, 2012).

The aim of this research is to study the activity of Gallic acid and Caffeic acid of honey on plasma membrane protein RCh1P of Candida species.

MATERIALS AND METHODS

Data

The 3D protein structure of plasma membrane protein RCh1P was not available in the protein data bank. Hence, the protein was modelled using the Swiss-Model online server program (https://swissmodel.expasy.org/). Template search with BLAST and HHblits has been performed against the SWISS-MODEL template library. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Phytochemicals identified from honey namely, caffeic acid, cinnamic acid, gallic acid, syringic acid, ellagic acid were selected for the study. The two-dimensional chemical structures in structured data format (SDF) were retrieved from PubChem database and were converted into Protein data bank (PDB) format using Pymol. Antifungal drug, fluconazole was processed similarly and considered as controls.

Interaction of the drug including pharmacological effects and mechanism of action. interaction with metabolic enzymes and transporters, influence on gene expression, possible adverse effects and side effects were predicted using Passonline web server (http://www.way2drug.com/passonline). SMILES (Simplified Molecular Input Line Entry System) of each drug was fed as the input for each drug. Ten interactions with the highest Pa value is shown in Table 1 and 2. Pa (probability "to be active") is an estimate of the probability of the drug belonging to the sub-class of active compounds resembling the established set of actives in the server training set. Similarly, Pi is the probability "to be inactive" that is estimated by the server.

The physiochemical properties, lipophilicity, water solubility, pharmacokinetics and drug-likeliness were assessed using the SwissADME online server program. SMILES of each drug was used as the input.

Optimal docking areas were calculated using molsoft online server (http://www.molsoft.com). The output was viewed using an Pymol Program.

Molecular Docking

Molecular docking was carried out using Hex Protein Docking server. The receptor and the ligand were fed as a .pdb file and the resulting interactions with the docking score were recorded.

RESULTS

The biological activity and drug-likeness of the selected phytochemicals were analysed using PASS Online and SwissADME tools, followed by molecular docking studies to evaluate their interaction with the plasma membrane protein RCh1P of Candida albicans. The results demonstrated promising inhibitory potential for both gallic acid and caffeic acid.

The prediction of biological activity using PASS Online revealed that gallic acid possesses a high probability of biological activity (Pa values >0.93) across several enzymatic targets. The compound showed strong predicted inhibitory activity against enzymes such as arylacetonitrilase, chlordecone reductase, dehydro-L-gulonate decarboxylase, testosterone 17β-dehydrogenase, and glutathione thiolesterase. The probability values (Pa) were significantly higher than the probability of inactivity (Pi), indicating a strong likelihood that gallic acid exhibits biologically relevant inhibitory effects. These findings suggest that gallic acid may interact with multiple biochemical pathways, thereby supporting its potential pharmacological relevance (Table 1).

The pharmacokinetic and physicochemical properties of gallic acid and caffeic acid were further evaluated using SwissADME. Both compounds exhibited favourable drug-likeness characteristics, including high gastrointestinal absorption and excellent water solubility. The topological polar surface area (TPSA) values were 97.99 Å² for gallic acid and 129.51 Å² for caffeic acid, which fall within acceptable ranges for oral bioavailability. Additionally, neither compound showed inhibitory activity against major cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), indicating a lower probability of metabolic drug interactions. Importantly, both molecules complied with Lipinski's rule of five with zero violations, further supporting their potential as drug-like compounds. The predicted bioavailability score of 0.56 for both molecules suggests moderate oral bioavailability, while the low synthetic accessibility scores indicate that these compounds are relatively easy to synthesize (Table 2).

Molecular docking analysis was carried out to examine the interaction of gallic acid and caffeic acid with the RCh1P plasma membrane protein of Candida albicans. The docking results revealed stable binding interactions between the phytochemicals and the target protein. Gallic acid demonstrated the strongest binding affinity, with an E-total docking score of −180.34, interacting with the amino acid residues GLN-145 and ILE-65. This highly negative docking energy

indicates a stable ligand–protein interaction, suggesting that gallic acid may effectively bind to and potentially inhibit the function of the RCh1P membrane protein.

Similarly, caffeic acid also exhibited a notable binding interaction with the protein, showing an E-total score of −158.74 and interacting with the amino acid residue ASN-184. Although the binding affinity was slightly lower than that of gallic acid, the negative docking score still indicates a favourable interaction between the compound and the target protein (Table 3).

Visualization of the docked complexes using PyMOL further confirmed the interaction patterns between the phytochemicals and the target protein. The binding residues observed in the docking analysis suggest that these compounds may interact with key functional regions of the plasma membrane protein, potentially interfering with its normal activity. Such interactions may disrupt essential cellular processes in Candida albicans, thereby contributing to the antifungal activity of these phytochemicals.

Table 1: Prediction of biological activity for Gallic acid

Pa	Pi	Activity
0,955	0,002	Arylacetonitrilase inhibitor
0,954	0,002	Chlordecone reductase inhibitor
0,950	0,002	Dehydro-L-gulonate decarboxylase inhibitor
0,950	0,003	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,944	0,002	Glutathione thiolesterase inhibitor
0,943	0,002	Alkane 1-monooxygenase inhibitor
0,941	0,003	Sugar-phosphatase inhibitor
0,938	0,002	NADPH-cytochrome-c2 reductase inhibitor
0,934	0,001	Threonine aldolase inhibitor
0,933	0,002	2-Hydroxyquinoline 8-monooxygenase inhibitor

Table 2: ADME properties of Gallic acid and Caffeic acid

ADME Parameters	Gallic acid	Caffeic acid
TPSA	97.99	129.51 Å²
Consensus Log Po/w	0.21	0.46
Water solubility	Very soluble	Very soluble

GI absorption	High	High
Inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4	No	No
Lipinkski (drug likeliness	0 violation	0 violation
Bioavailability score	0.56	0.56
Synthetic accessibility	1.22	2.46

Table 3: Docking results of the compounds

S.No	Phytochemicals	PubChem ID	Binding Residues	E-total
1	Gallic acid	370	GLN-145 and ILE 65	-180.34
2	Caffeic acid	689043	ASN-184	-158.74

Figure 1: **Docked complex of the compounds**

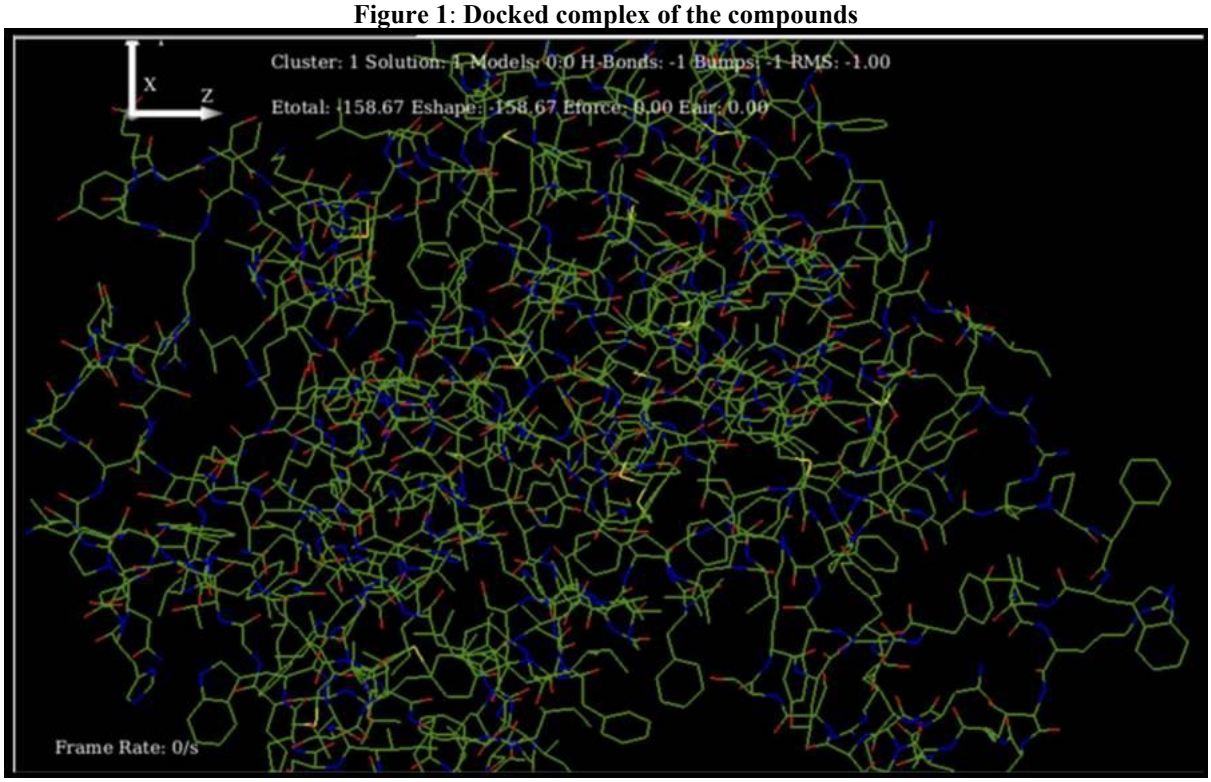


Figure 2: **Molecular docking of Gallic acid on RCh1P membrane of C. albicans**

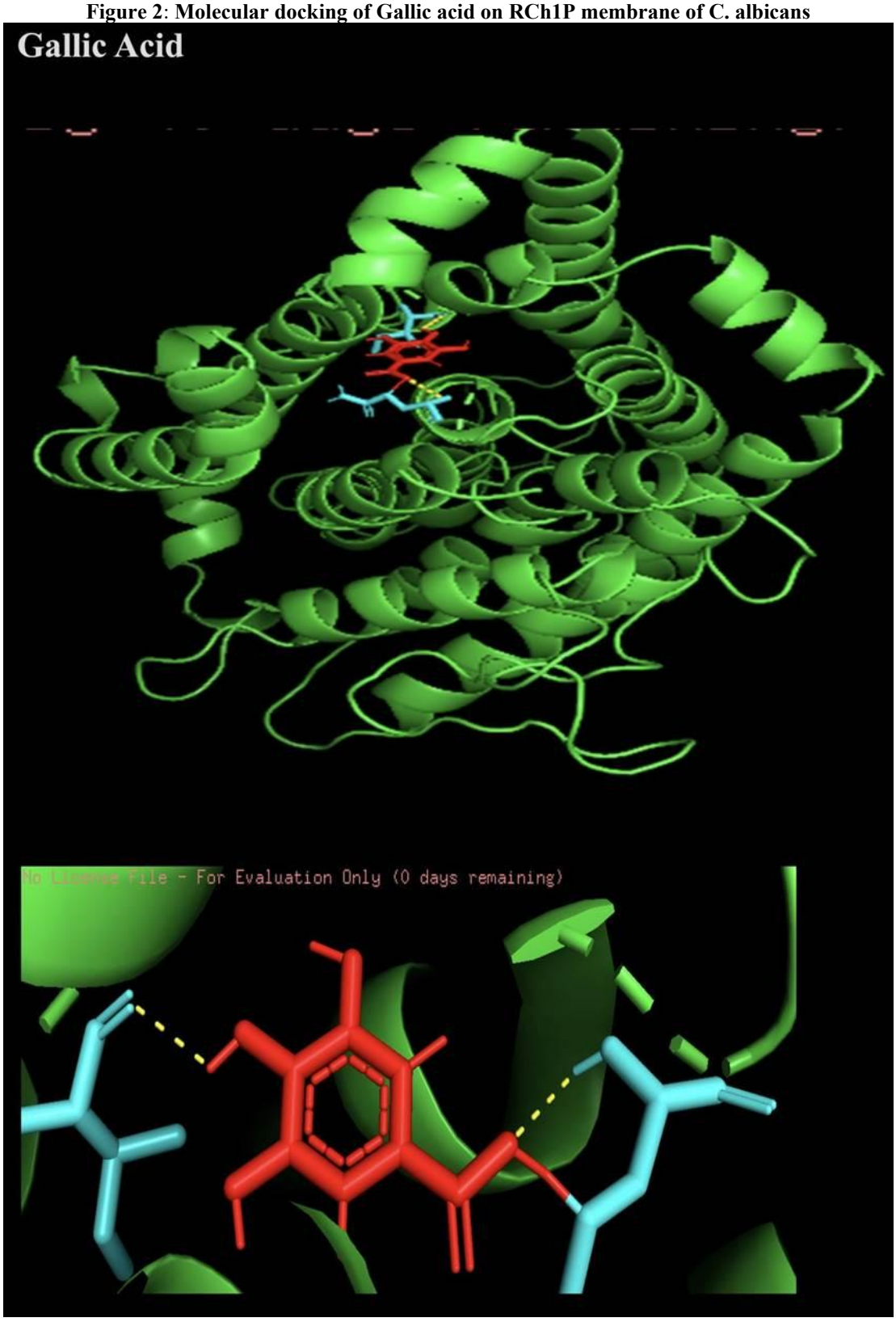
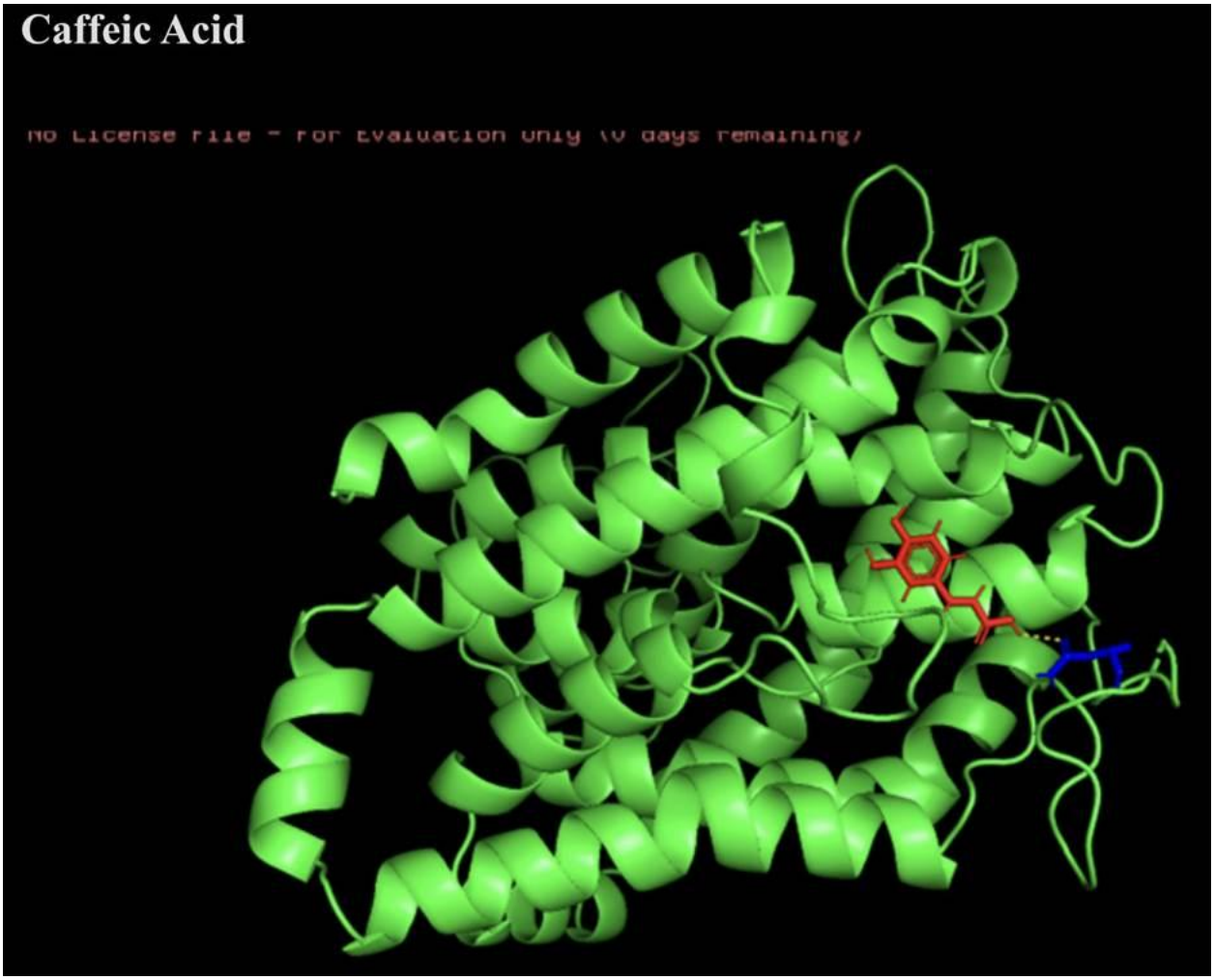


Figure 3: **Molecular docking of Caffeic acid on RCh1P membrane of C. albicans**



DISCUSSION

There are lots of beneficial effects of Gallic acid and Caffeic acid and are also provided by studies. Caffeic acid protected human KF1 diploid fibroblast and A431 epidermoid carcinoma cell lines from UVC-induced cytotoxicity(Rodrigues et al., 2015). It was shown to inhibit UVB (280–320 nm) radiation-induced IL-10 expression and the activation of the mitogen-activated protein kinases (MAPKs) in mouse skin(Kilani-Jaziri et al., 2017). In recent work, it was shown caffeic acid inhibited the cell proliferation of HepG2 cells in a dose-dependent manner. It blocked the MMP-9 expression by inhibiting the NF-κB activity(Agunloye et al., 2019). Finally, they showed that caffeic acid at a dose of 20 mg/kg retarded the growth of HepG2 tumor xenografts in immunosuppressive mice(Nagaoka et al., 2002). Caffeic acid was shown to inhibit the growth of HT 29 colon cancer cells. Caffeic acid at a concentration of 2500 μM found to inhibit 50% cell proliferation of HT 29 cells(Xie et al., 2017). However, no further work was initiated about the apoptosis induced by caffeic acid in colon cancer cells(Yang et al., 2013).

Through a variety of molecular mechanisms of action on the cell cycle, cell apoptotic processes, angiogenesis, invasion, and metastasis, gallic acid obtained from natural dietary sources has anticarcinogenic properties. These effects could be mostly caused by a particular impact on ADAM17, ATM kinase, Bax/Bcl-2, COX, ribonucleotide reductase, UGDH, NF-B, and vegf/vegfR signalling pathways(Ibrahim et al., 2026). In addition to its well-established application in medication development, gallic acid is added to food supplements to reduce the risk of cancer(Su et al., 2026). Nonetheless, more fundamental, preclinical, and clinical studies on gallic acid might offer a path forward for its eventual use in the field of cancer chemoprevention(S. Verma et al., 2013).

Overall, the docking analysis suggests that gallic acid exhibits stronger inhibitory potential compared to caffeic acid, based on the lower docking energy and multiple binding interactions with the target protein (Gunasekaran & Sathishkumar, 2026; Hameed et al., 2024; Yuwanati et al., 2026). These findings indicate that both compounds derived from honey possess promising antifungal properties and may contribute to inhibiting the growth or survival of Candida albicans through interaction with the RCh1P plasma membrane protein(Jayaraman et al., 2026; Venkatesan & Sathishkumar, 2026).

CONCLUSION

We may conclude from this study that the effect of Gallic acid and Caffeic acid on plasma membrane protein RCh1P is inhibitory. Hence these phytochemicals stop the growth of plasma membrane protein RCh1P and prevent extensive invasion of Candidiasis.

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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