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Comparative Evaluation of Quercetin, Naringenin, and Resveratrol Against *Malassezia furfur* Through Molecular Docking and *In vitro* Antifungal Studies

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ABSTRACT

Objective: Malassezia furfur is a lipid-dependent yeast associated with seborrheic dermatitis and other superficial fungal infections. Although natural polyphenols possess diverse biological activities, their antifungal efficacy against M. furfur remains inadequately explored. The present study investigated the antifungal potential of quercetin, naringenin, and resveratrol through an integrated computational and experimental approach. **Methods:** Molecular docking was performed using AutoDock Vina to evaluate the interactions of the selected phytoconstituents with lanosterol 14-alpha demethylase (CYP51; PDB ID: 5V5Z), with ketoconazole used as the reference antifungal agent. UV–Visible and Fourier-transform infrared (FT-IR) spectroscopy were used to confirm the identity and structural integrity of the investigated compounds prior to biological evaluation. Antifungal activity was subsequently observed using agar well diffusion and disc diffusion assays. **Results:** Molecular docking demonstrated that quercetin exhibited the highest binding affinity among the investigated phytoconstituentsfollowed by naringenin and resveratrolwhereas ketoconazole showed the strongest overall interaction. Spectroscopic analyses confirmed the expected structural characteristics of all compounds. Although all three phytoconstituents interacted with the CYP51 binding site, the in vitro studies demonstrated only limited antifungal activity, and increasing compound concentration produced minimal improvement when compared with ketoconazole. The computational and experimental findings followed a similar ranking pattern; however, favourable molecular docking interactions did not translate into comparable biological efficacy. These findings demonstrate that ligand binding alone is insufficient to predict antifungal activity and highlight the importance of experimental validation when evaluating potential antifungal agents. **Conclusion:** The present study provides a comparative assessment of three naturally occurring polyphenols and offers a basis for future investigations involving structural modification, formulation development, and improved drug delivery strategies to enhance antifungal efficacy.

INTRODUCTION:

Skin disorders arise through the combined effects of microbial colonization, oxidative imbalance, and inflammatory responses, all of which contribute to tissue damage and disease progression. Extraformation of reactive oxygen species (ROS) disrupts cellular homeostasis, weakens the protective function of the skin barrier, and increases susceptibility to injury, whereas sustained inflammation further exacerbates irritation and pathological changes. Consequently, successful management of many dermatological conditions

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requires therapeutic strategies that can effectively suppress microbial growth while simultaneously limiting inflammatory processes [1–3].Among inflammatory skin diseases, seborrheic dermatitis is a chronic relapsing disorder that predominantly affects sebum-rich regions such as the scalp, face, and upper trunk [4]. It commonly presents with erythema, scaling, pruritus, and recurrent episodes of irritation. The disease develops through a multifactorial process involving alterations in sebum secretion, impairment of the skin barrier, host immune responses, and microbial colonization [4]. Among the microorganisms associated with seborrheic dermatitis, species belonging to the genus Malassezia are considered major contributors to disease development [5–7]. Malassezia furfur is a lipid-dependent yeast that normally exists as part of the resident skin microbiota but may become pathogenic under favourable conditions by metabolizing host lipids and generating metabolites capable of triggering inflammatory responses [5,6]. This adaptation promotes persistent colonization of lipid-rich skin surfaces and contributes to disease progression [6,7]. The lipid dependence of M. furfur also creates unique challenges during antifungal susceptibility testing, distinguishing it from many other fungal pathogens [7,8]. In particular, lipid supplementation required for laboratory cultivation can influence the diffusion, distribution, and apparent activity of antifungal compounds during experimental evaluation [8].Given the important role of M. furfur in the pathogenesis of seborrheic dermatitis, antifungal therapy continues to represent a fundamental component of clinical management. Current therapeutic approaches generally combine antifungal agents with treatments aimed at reducing inflammation and scaling [4,9]. Among the available antifungal drugs, ketoconazole is mostly used agent for the treatment of Malassezia-associated infections [10–12]. Its antifungal activity results primarily from inhibition of lanosterol 14-alpha demethylase (CYP51), an important enzyme for fungal biosynthesis [10–13]. Suppression of ergosterol production disrupts fungal cell membrane integrity, ultimately impairing fungal growth and survival [10–12]. Although ketoconazole has demonstrated proven clinical efficacy, frequent disease recurrence and other therapeutic limitations continue to encourage the identification of alternative antifungal agents [14,15]. As a result, considerable research has focused on discovering novel compounds capable of targeting molecular pathways essential for fungal survival [30]. Among these molecular targets, CYP51 has received significant attention because of its indispensable role in maintaining fungal viability [12,13]. In parallel, molecular docking isa efficient computational strategy for predicting ligand–protein interactions, estimating binding affinity, and identifying critical amino acid residues involved in molecular recognition before experimental validation [16,17]. Such computational analyses provide valuable preliminary information regarding the possible mechanisms of action of prospective antifungal compounds [17].Natural products have long played a central role in the discovery of therapeutic agents and remain an important source of structurally diverse bioactive molecules [18,19]. Numerous phytochemicals exhibit a wide range of pharmacological activities, making them attractive candidates for the development of novel therapies against various diseases [18,19,31]. Among these naturally occurring compounds, polyphenols have attracted particular interest because of their broad biological activities together with favourable safety profiles [2,3,8]. These secondary metabolites are widely distributed in fruits, vegetables, medicinal plants, and other dietary sources, where they contribute to plant defence mechanisms and normal physiological functions [18,19].Among naturally occurring polyphenols, quercetin, naringenin, and resveratrol have been investigated extensively owing to their diverse pharmacological properties. Quercetin is a flavonoid abundantly present in onions, apples, berries, and numerous medicinal plants [18–20]. Naringenin is predominantly found in citrus fruits as a representative flavanone, whereas resveratrol is a naturally occurring stilbene commonly present in grapes, peanuts, and red wine [1,2,18]. Despite belonging to different chemical classes, these phytoconstituents share several biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [1–3]. Their capacity to regulate multiple cellular pathways has stimulated growing interest in their potential application for the treatment of infectious and inflammatory disorders [1–3]. Because of these reported biological properties, they represent promising candidates for the development of alternative therapeutic approaches against Malassezia-associated skin infections [1–3,32]. Prior to biological evaluation, however, confirmation of compound identity and structural integrity is essential to ensure the reliability of experimental findings. For this purpose, UV–Visible spectroscopy and Fourier-transform infrared (FT-IR) spectroscopy are routinely employed for physicochemical characterization and structural confirmation of phytochemicals [21,22]. These analytical techniques help verify that the observed biological effects originate from the intended compounds rather than impurities or degradation products [21,22].

Although quercetin, naringenin, and resveratrol have been widely investigated for their biological activities, studies directly comparing their antifungal potential against Malassezia furfur in combination with molecular docking analysis targeting CYP51

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remain limited. Therefore, the present study was designed to comprehensively investigate the antifungal potential of these three phytoconstituents through physicochemical characterization, molecular docking analysis targeting CYP51, and in vitro antifungal evaluation. Furthermore, the computational predictions were compared with the experimental findings to obtain a more comprehensive understanding of the antifungal potential of these naturally occurring polyphenols.

OBJECTIVE OF THE STUDY

The present study aimed to comprehensively evaluate the antifungal potential of quercetin, naringenin, and resveratrol against Malassezia furfur using an integrated computational and experimental approach. Molecular docking was performed to investigate the interactions of the selected phytoconstituents with lanosterol 14-alpha demethylase (CYP51). The compounds were subsequently characterized using UV–Visible and Fourier-transform infrared (FT-IR) spectroscopic analyses to confirm their identity and structural integrity. Their antifungal activities were then evaluated using agar well diffusion and disc diffusion methods and compared with ketoconazole as the reference antifungal agent.

MATERIALS AND METHODS:

Chemicals:

Quercetin, resveratrol, and naringenin were procured from Yucca Pharmaceuticals, Mumbai, India. Ketocip 1% w/v ketoconazole shampoo (Cipla Ltd., Mumbai, India) was used as the reference antifungal agent. Dimethyl sulfoxide (DMSO) served as both the solvent and negative control. Corn oil was used as a lipid supplement in the culture medium to support the growth of the lipophilic fungus Malassezia furfur.

Molecular Docking Study

Molecular docking was performed to evaluate the binding interactions of quercetin, resveratrol, and naringenin with lanosterol 14-alpha demethylase (CYP51), a key enzyme involved in fungal ergosterol biosynthesis. The crystal structure of CYP51 (PDB ID: 5V5Z) was retrieved from the Protein Data Bank [13]. The three-dimensional structures of the selected phytoconstituents were prepared and geometrically optimized using Avogadro prior to docking analysis [25]. Format conversion was performed using Open Babel [21], followed by energy minimization of the optimized ligand structures. Molecular docking simulations were subsequently carried out using AutoDock Vina [16]. Binding affinity was evaluated based on the calculated binding energy (kcal/mol), where more negative values indicate stronger ligand–protein interactions. The docked ligand–protein complexes were visualized using UCSF Chimera, and the molecular interactions were analysed using BIOVIA Discovery Studio Visualizer [26] to identify hydrogen bonds, hydrophobic interactions, and other non-covalent interactions within the binding site.

Physicochemical Characterization

UV–Visible Spectroscopy

UV–Visible spectroscopic analysis of quercetin, resveratrol, and naringenin was performed using a Shimadzu UV-1800 spectrophotometer over a wavelength range of 200–800 nm. Methanol was used as the solvent, and measurements were recorded using a 1 cm quartz cuvette. The absorption maxima (λmax) obtained for quercetin, resveratrol, and naringenin were 372, 306, and 289 nm, respectively. The obtained absorption maxima (λmax) were compared with reported literature values to verify the identity of the investigated compounds prior to further analyses [1–3,18].

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra were recorded over the range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1} using 32 scans for each sample. The spectra were evaluated to identify the characteristic functional groups present in quercetin, resveratrol, and naringenin [21,22]. The observed absorption bands corresponded to the characteristic functional groups and expected chemical structures of the investigated compounds, thereby confirming their structural integrity prior to biological evaluation.

Fungal Strain and Culture Conditions

Malassezia furfur MTCC 1374 was procured from the Microbial Type Culture Collection (MTCC), CSIR–Institute of Microbial Technology (IMTECH), Chandigarh, India. The fungal strain was maintained on Sabouraud Dextrose Agar (SDA) supplemented with corn oil to provide the lipid requirement necessary for fungal growth. Cultures were incubated at 37°C for 48–72 h to obtain actively growing fungal colonies for subsequent experiments.

Inoculum Preparation

All glassware and Petri plates were sterilized in a hot-air oven at 200°C for 30 min prior to use. Sterile distilled water was used throughout the experimental procedure. A fresh culture of Malassezia furfur was suspended in sterile distilled water to prepare the inoculum. Sabouraud Dextrose Agar (SDA) was prepared under aseptic conditions and poured into sterile Petri plates. After solidification, 2–3 drops of corn oil were applied to the agar surface to support fungal growth. The prepared inoculum was evenly spread over the agar surface, and the plates were

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incubated at 37°C for 48 h prior to antifungal susceptibility testing [24].

Antifungal Susceptibility Testing

Agar Well Diffusion Method

Antifungal activity was evaluated using the agar well diffusion method [24]. SDA plates supplemented with corn oil were inoculated with Malassezia furfur, and wells were prepared using a sterile cork borer. Quercetin, naringenin, and resveratrol were initially evaluated at concentrations of 125, 250, 500, and 1000 µg/mL. To investigate the effect of increasing concentration on antifungal activity, additional experiments were performed at concentrations of 1000, 2000, 4000, and 8000 µg/mL. Ketoconazole shampoo was used as the positive control, whereas DMSO served as the negative control. Following incubation at 37°C for 48–72 h, the diameters of the inhibition zones were measured in millimetres. All experiments were performed in duplicate, and the results were expressed as mean ± standard deviation (SD).

Disc Diffusion Method

Antifungal activity was further evaluated using the disc diffusion method [25]. Sterile paper discs (6 mm diameter) were impregnated with the respective test solutions and placed on SDA plates previously inoculated with Malassezia furfur. The plates were incubated at 37°C for 48–72 h, after which antifungal activity was determined by measuring the diameters of the inhibition zones surrounding each disc. All experiments were performed in duplicate, and the results were expressed as mean ± standard deviation (SD).

RESULTS:

The results obtained from the computational, analytical, and experimental investigations are presented in the following sections. Molecular docking analysis was initially performed to evaluate the binding interactions of quercetin, naringenin, and resveratrol with the CYP51 target protein. This was followed by physicochemical characterization using UV–Visible and FT-IR spectroscopy to confirm the identity and structural integrity of the selected phytoconstituents. Subsequently, the antifungal activities of the compounds were evaluated using agar well diffusion and disc diffusion assays. Finally, the computational predictions were compared with the experimental findings to provide a comprehensive assessment of the antifungal potential of the investigated phytoconstituents.

Molecular Docking Analysis

Target Selection and Docking Rationale

Lanosterol 14-alpha demethylase (CYP51) was selected as the molecular target because of its essential role in fungal ergosterol biosynthesis. Ergosterol is a major structural component of the fungal cell membrane, and inhibition of CYP51 disrupts membrane integrity, thereby impairing fungal growth and survival [10–13]. Consequently, CYP51 has become one of the most extensively investigated molecular targets in antifungal drug discovery [12,13]. To evaluate the theoretical antifungal potential of the selected phytoconstituents, molecular docking studies were performed using the crystal structure of CYP51 (PDB ID: 5V5Z). Quercetin, naringenin, and resveratrol were docked into the active site of the enzyme and compared with ketoconazole, a well-established CYP51 inhibitor used as the reference antifungal agent. Binding affinity was evaluated based on the calculated binding energy (kcal/mol), where more negative values indicate stronger predicted ligand–protein interactions [16,17]. The calculated binding energies of the investigated compounds are presented in Table 1.

TABLE 1. Binding energy (kcal/mol) of test compounds with CYP51 (PDB ID: 5V5Z)

Compound	Binding energy (kcal/mol)
Ketoconazole	-10.7
Quercetin	-8.7
Naringenin	-8.1
Resveratrol	-7.5

Among the investigated compounds, ketoconazole exhibited the strongest predicted binding affinity toward CYP51. All three phytoconstituents also demonstrated favourable interactions within the enzyme binding pocket, although differences in binding strength were observed.

Molecular Docking Results and Binding Affinity Analysis

Molecular docking analysis demonstrated that all three investigated phytoconstituents could interact with the active site of CYP51, although with varying binding affinities. The calculated binding energies revealed differences in the predicted strength of ligand–protein interactions among the investigated compounds (Table 1). Ketoconazole exhibited the lowest binding energy (−10.7 kcal/mol), indicating the strongest predicted interaction with CYP51. Among the phytoconstituents, quercetin demonstrated the most favourable binding affinity (−8.7 kcal/mol), followed by naringenin (−8.1 kcal/mol) and resveratrol (−7.5 kcal/mol). The observed ranking indicates that quercetin possessed the highest predicted affinity for the CYP51 active site among the investigated natural compounds, whereas ketoconazole remained the highest-affinity ligand overall. Despite differences in binding strength, all three phytoconstituents demonstrated measurable interactions within the CYP51 binding region, supporting their suitability for subsequent experimental evaluation.

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Amino Acid Interaction and Binding Mode Analysis

To further characterize ligand binding within the CYP51 active site, the docked complexes were analysed to identify the amino acid residues involved in molecular interactions. The interaction profiles of ketoconazole, quercetin, naringenin, and resveratrol are summarized in Table 2, while the corresponding two-dimensional interaction diagrams are presented in Figure 1(a–d).

TABLE 2. Amino acid interactions and binding profile of test compounds with CYP51.

Ligand	Binding Energy (kcal/mol)	Hydrogen Bonds	π–π / π–Alkyl Interactions	Other Interactions
Ketoconazole	-10.7	HIS A:377, SER A:378	TYR A:118, PHE A:233, PHE A:380, LEU A:300, LEU A:139	GLY A:303, GLY A:307, GLY A:308, ILE A:131, ILE A:304, MET A:508, CYS A:470, LEU A:121
Quercetin	-8.7	HIS A:377	PHE A:233, LEU A:87, PRO A:230	TYR A:64, MET A:508, SER A:507, SER A:378, LEU A:376, ILE A:379, PHE A:380
Naringenin	-8.1	Unfavourable interaction with HIS A:377	PHE A:233, LEU A:87	TYR A:64, TYR A:118, SER A:378, LEU A:376, MET A:508, VAL A:509, VAL A:510
Resveratrol	-7.5	SER A:507, MET A:508	PRO A:230, LEU A:87, LEU A:88	TYR A:64, PHE A:233, PHE A:380, GLY A:65, HIS A:377, ILE A:231

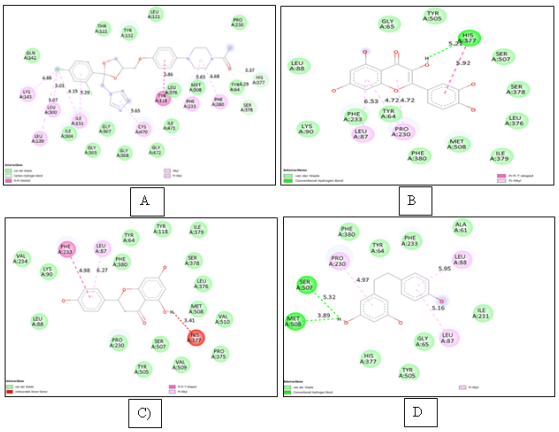

FIGURE 1. Two-dimensional interaction diagrams of ligands with CYP51 (PDB ID: 5V5Z): (a) Ketoconazole, (b) Quercetin, (c) Naringenin, and (d) Resveratrol.

Comparative analysis demonstrated distinct interaction patterns among the investigated ligands within the CYP51 binding pocket. Ketoconazole exhibited the most extensive interaction network, comprising hydrogen bonds, aromatic π interactions, π–alkyl interactions, and multiple hydrophobic contacts, consistent with its highest binding affinity. Among the investigated phytoconstituents, quercetin established favourable hydrogen-bonding, aromatic, and hydrophobic interactions that were associated with its comparatively stronger predicted binding affinity. Naringenin also formed several stabilizing interactions within the active site; however, an unfavourable interaction involving HIS A:377 was observed. Resveratrol interacted with CYP51 through hydrogen bonds together with π–alkyl and hydrophobic interactions but formed comparatively fewer stabilizing contacts than quercetin.Several amino acid residues, including HIS A:377 and PHE A:233, were involved in the binding of more than one ligand, indicating their importance within the CYP51 binding pocket. Overall, the interaction profiles were consistent with the calculated binding energies, and the predicted binding affinity followed the order: Ketoconazole > Quercetin > Naringenin > Resveratrol.

The results obtained from the computational, analytical, and experimental investigations are presented in the following sections. Molecular docking analysis was first performed to evaluate the binding interactions of quercetin, naringenin, and resveratrol with the CYP51 target protein. This was followed by physicochemical characterization using UV–Visible and FT-IR spectroscopy to confirm the identity and structural integrity of the selected phytoconstituents. Subsequently, the antifungal activities of the compounds were evaluated using agar well diffusion and disc diffusion assays. The computational and experimental findings were then examined to provide a comprehensive assessment of the antifungal potential of the investigated phytoconstituents.

Physicochemical Characterization

UV–Visible Spectroscopy Analysis

UV–Visible spectroscopy was performed to confirm the identity and characteristic spectral properties of quercetin, naringenin, and resveratrol prior to biological evaluation. The recorded spectra exhibited well-defined absorption maxima corresponding to the characteristic chromophoric

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systems of the investigated phytoconstituents. Quercetin showed a prominent absorption maximum (λ_{max}) at 372 nm, whereas resveratrol and naringenin exhibited λ_{max} values of 306 and 289 nm, respectively. The observed absorption maxima were in close agreement with previously reported literature values, confirming the identity and spectral integrity of the investigated compounds [1–3,18]. Furthermore, no unexpected absorption peaks or significant spectral shifts were observed, indicating the absence of major impurities or detectable degradation prior to subsequent computational and biological investigations.

Fourier Transform Infrared (FT-IR) Spectral Analysis

FT-IR spectroscopy was performed to identify the characteristic functional groups present in the investigated phytoconstituents and to verify their structural integrity before antifungal evaluation.The FT-IR spectrum of quercetin (Figure 2) exhibited a broad absorption band at 3457–3427 cm^{-1} corresponding to O–H stretching vibrations of phenolic hydroxyl groups. Absorption bands at 2984 and 2934 cm^{-1} were assigned to C–H stretching vibrations, while the region between 1640 and 1600 cm^{-1} represented conjugated carbonyl (C=O) and aromatic C=C stretching vibrations. Additional peaks observed at 1418 cm^{-1} were attributed to O–H bending and aromatic ring vibrations, whereas bands at 1254, 1133, and 1022 cm^{-1} corresponded to C–O stretching vibrations of phenolic functional groups.

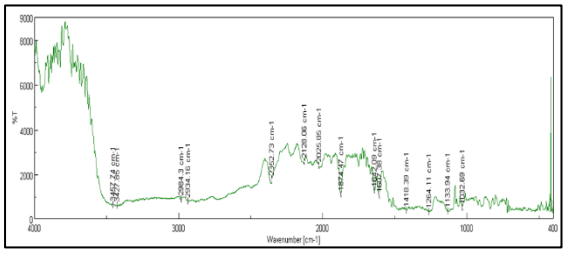

FIGURE 2. FT-IR spectrum of quercetin (KBr pellet method).

The FT-IR spectrum of naringenin (Figure 3) displayed a broad absorption band at 3355–3255 cm^{-1}, characteristic of phenolic O–H stretching vibrations. Peaks at 2988 and 2880 cm^{-1} corresponded to C–H stretching vibrations, while a distinct absorption band near 1633 cm^{-1} indicated the presence of the carbonyl group characteristic of the flavanone structure. Aromatic C=C stretching vibrations were observed between 1612 and 1505 cm^{-1}, whereas peaks at 1377 and 1161 cm^{-1} were assigned to C–O stretching vibrations. Additional absorption bands in the region of 1038–983 cm^{-1} further confirmed the presence of phenolic functional groups.

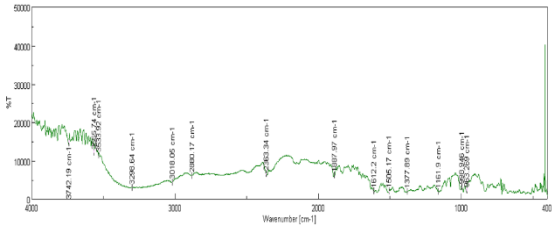

FIGURE 3. FT-IR spectrum of naringenin (KBr pellet method).

The FT-IR spectrum of resveratrol (Figure 4) exhibited a broad absorption band around 3319 cm^{-1} corresponding to phenolic O–H stretching vibrations. A characteristic absorption peak near 3007 cm^{-1} was attributed to aromatic C–H stretching vibrations, whereas strong absorption bands between 1601 and 1589 cm^{-1} represented aromatic C=C stretching vibrations. A prominent peak at approximately 966 cm^{-1} corresponded to the trans –CH=CH– out-of-plane bending vibration characteristic of stilbene derivatives. Unlike quercetin and naringenin, no carbonyl stretching band was observed. Additional absorption bands between 1513 and 1387 cm^{-1} together with the band around 1150 cm^{-1} were assigned to aromatic ring vibrations and C–O stretching vibrations, respectively, confirming the characteristic structural features of resveratrol.

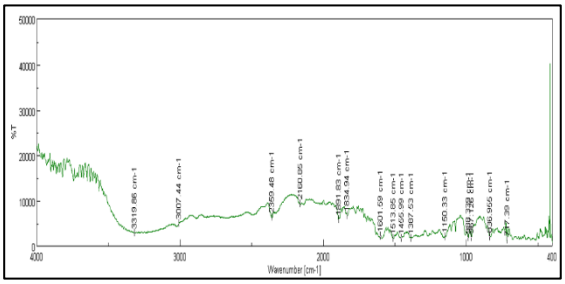

FIGURE 4. FT-IR spectrum of resveratrol (KBr pellet method).

Experimental Antifungal Activity

Agar Well Diffusion

The antifungal activities of quercetin, naringenin, and resveratrol against Malassezia furfur were evaluated using the agar well diffusion method at concentrations ranging from 125 to 1000 µg/mL. The results are presented in Table 3.

TABLE 3. Antifungal activity of test compounds against Malassezia furfur using the agar well diffusion method at concentrations of 125–1000 µg/mL (mean ± SD, n = 2).

Concentration / Compound	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
Ketoconazole (STD)	26.23 ± 0.04	25.85 ± 4.45	22.45 ± 6.72	16.20 ± 0.71
Quercetin	34.75 ± 2.47	30.85 ± 2.33	18.25 ± 4.60	13.45 ± 2.47
Naringenin	14.35 ± 0.21	11.25 ± 0.35	14.60 ± 7.21	11.60 ± 1.27

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Resveratrol	10.73 ± 0.67	11.50 ± 0.71	9.63 ± 0.88	10.75 ± 0.35
DMSO (Control)	12.50 ± 2.12	12.50 ± 2.12	12.50 ± 2.12	12.50 ± 2.12

The agar well diffusion assay demonstrated measurable inhibitory activity for all investigated phytoconstituents; however, considerable differences were observed in the extent of fungal growth inhibition. Among the tested compounds, quercetin consistently produced the largest zones of inhibition, with the maximum inhibition observed at 125 µg/mL (34.75 ± 2.47 mm), followed by 250 µg/mL (30.85 ± 2.33 mm). At higher concentrations (500 and 1000 µg/mL), comparatively smaller inhibition zones of 18.25 ± 4.60 mm and 13.45 ± 2.47 mm, respectively, were recorded. Naringenin exhibited moderate antifungal activity, with inhibition zones ranging from 11.25 ± 0.35 mm to 14.60 ± 7.21 mm, whereas resveratrol demonstrated comparatively lower inhibitory activity throughout the investigated concentration range. As expected, ketoconazole, the reference antifungal agent, produced the largest inhibition zones, whereas DMSO (negative control) exhibited no measurable antifungal activity. Overall, quercetin demonstrated the greatest antifungal activity among the investigated phytoconstituents under the experimental conditions employed.

Disc Diffusion

The antifungal activities of quercetin, naringenin, and resveratrol were further evaluated using the disc diffusion method at concentrations ranging from 1000 to 8000 µg/mL. The results obtained from duplicate experiments are presented in Table 4.

TABLE 4. Antifungal activity of test compounds against Malassezia furfur using the disc diffusion method at concentrations of 1000–8000 µg/mL (mean ± SD, n = 2).

Concentration / / Compound	**1000 µg/mL**	**2000 µg/mL**	**4000 µg/mL**	**8000 µg/mL**
Ketoconazole (STD)	37.50 ± 5.89	27.98 ± 7.52	30.83 ± 3.54	24.17 ± 2.59
Quercetin	8.67 ± 3.77	8.67 ± 3.77	8.17 ± 2.59	6.00 ± 0.00
Naringenin	14.83 ± 4.00	9.83 ± 5.90	7.27 ± 1.79	6.00 ± 0.00
Resveratrol	13.83 ± 3.99	7.50 ± 2.59	6.00 ± 0.00	6.00 ± 0.00
DMSO (Control)	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

Compared with the agar well diffusion assay, the disc diffusion method produced generally smaller inhibition zones for all investigated phytoconstituents. Overall, all three phytoconstituents exhibited comparatively weak inhibitory activity in the disc diffusion assay. Although naringenin and resveratrol produced measurable inhibition at the lower tested concentrations, the inhibition zones remained small throughout the investigated concentration range, while quercetin exhibited only modest inhibitory activity. In contrast, ketoconazole consistently produced the largest inhibition zones at all tested concentrations, confirming its superior antifungal efficacy under the experimental conditions employed. For several concentrations, the measured inhibition zones remained close to the diameter of the paper disc, indicating limited antifungal activity of the investigated phytoconstituents when evaluated using the disc diffusion technique.

Effect of Concentration on Antifungal Activity

To further investigate the influence of concentration on antifungal activity, the selected phytoconstituents were evaluated at concentrations ranging from 1000 to 8000 µg/mL using the disc diffusion method. The comparative results are illustrated in Figure 7.Increasing the concentration of the investigated phytoconstituents from 1000 to 8000 µg/mL did not result in a proportional increase in the diameter of the inhibition zones. Although minor variations were observed among the investigated compounds, the overall antifungal activity remained limited throughout the tested concentration range. Even at the highest concentration (8000 µg/mL), the inhibition zones produced by quercetin, naringenin, and resveratrol remained substantially smaller than those produced by ketoconazole. These findings suggest that increasing concentration alone was insufficient to substantially improve the antifungal activity of the investigated polyphenols under the experimental conditions employed.

FIGURE 5. Agar well diffusion assay plates showing the antifungal activity of quercetin, naringenin, and resveratrol against Malassezia furfur. Uppercase letters (Q, R, and N) represent Batch I, whereas lowercase letters (q, r, and n) represent Batch II (independent duplicate batch). For Batch I, Q1, R1, and N1 represent 1000 µg/mL, whereas Q2, R2, and N2 represent 500 µg/mL. For Batch II, q1, r1, and n1 represent 1000 µg/mL; q2, r2, and n2 represent 500 µg/mL; q3, r3, and n3 represent 250 µg/mL; and q4, r4, and n4 represent 125 µg/mL. S1 denotes the ketoconazole standard.

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FIGURE 6. Disc diffusion assay plates showing the antifungal activity of quercetin, naringenin, and resveratrol against Malassezia furfur. Uppercase letters (Q, R, and N) represent Batch I, whereas lowercase letters (q, r, and n) represent Batch II (independent duplicate batch). For Batch I, Q1, R1, and N1 represent 8000 µg/mL, whereas Q2 and R2 represent 4000 µg/mL. For Batch II, q1, r1, and n1 represent 8000 µg/mL, whereas q2 and r2 represent 4000 µg/mL. S1 and s1 denote the ketoconazole standard. and DMSO = negative control.

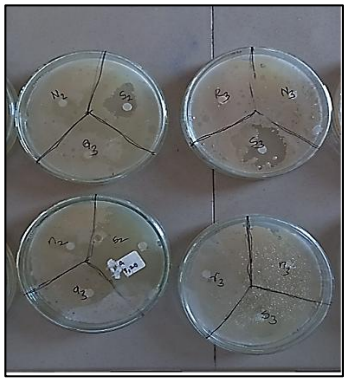

FIGURE 7. Disc diffusion assay plates showing the antifungal activity of quercetin, naringenin, and resveratrol against Malassezia furfur at higher concentrations. Uppercase letters (Q, R, N, and S) represent Batch I, whereas lowercase letters (q, r, n, and s) represent Batch II (independent duplicate batch). For Batch I, N2 represents 4000 µg/mL, Q3, R3, and N3 represent 2000 µg/mL, and S2 and S3 denote the ketoconazole standard. For Batch II, n2 represents 4000 µg/mL, q3, r3, and n3 represent 2000 µg/mL, and s2 and s3 denote the ketoconazole standard.

DISCUSSION

The present study integrated molecular docking, physicochemical characterization, and in vitro antifungal evaluation to investigate the antifungal potential of quercetin, naringenin, and resveratrol against Malassezia furfur. The discussion interprets the computational and experimental findings collectively, with particular emphasis on the relationship between predicted ligand–protein interactions and the observed biological activity. The findings also highlight the importance of experimental validation when assessing the therapeutic potential of naturally occurring phytoconstituents.

Structure–Activity Relationship (SAR) and Molecular Docking

Comparative molecular docking analysis revealed noticeable differences in the interactions of quercetin, naringenin, and resveratrol with lanosterol 14-alpha demethylase (CYP51). Among the investigated phytoconstituents, quercetin showed the most favourable docking score (−8.7 kcal/mol), followed by naringenin (−8.1 kcal/mol) and resveratrol (−7.5 kcal/mol), whereas ketoconazole exhibited the strongest binding affinity (−10.7 kcal/mol). The variation in docking scores appears to be associated with the structural characteristics of the investigated compounds and their ability to establish stabilizing interactions within the active site of CYP51. Quercetin interacted with key amino acid residues, including HIS A:377 and PHE A:233, through hydrogen-bonding and hydrophobic interactions, which may have enhanced the stability of the docked complex. Although naringenin occupied a similar binding region, its interaction profile was comparatively less extensive. Resveratrol also interacted with residues within the active site but formed fewer stabilizing contacts, which may account for its lower predicted affinity. As expected, ketoconazole exhibited the most comprehensive interaction network, consistent with its established role as a CYP51 inhibitor. These observations suggest that the quality and distribution of ligand–protein interactions influence the predicted affinity of compounds toward CYP51. Nevertheless, docking analysis represents only a computational prediction of molecular recognition and should be interpreted as a preliminary screening tool rather than direct evidence of biological efficacy. Consequently, experimental validation remains essential to determine the practical significance of these predicted interactions.

Physicochemical Characterization

The analytical characterization confirmed that the investigated phytoconstituents possessed the expected physicochemical properties before biological evaluation. The UV–Visible absorption maxima obtained for quercetin, naringenin, and resveratrol were in agreement with published literature, while FT-IR spectra displayed the characteristic functional groups associated with each compound without indicating major degradation or contamination [1,18–20]. These observations verified the identity and structural integrity of the investigated phytoconstituents and ensured that the subsequent computational and experimental findings reflected the properties of the intended compounds rather than variations in sample quality.

Comparison of Agar Well and Disc Diffusion Assays

Evaluation of antifungal activity using both agar well diffusion and disc diffusion assays demonstrated a consistent trend, although the extent

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of inhibition differed between the two methods. In both assays, quercetin produced greater inhibition than naringenin and resveratrol, whereas ketoconazole remained the most effective antifungal agent. However, inhibition zones obtained by the agar well diffusion method were consistently larger than those observed in the disc diffusion assay. This difference is likely attributable to the manner in which the compounds diffuse through the agar medium. In the agar well diffusion technique, the test solution is delivered directly into the agar well, allowing a larger amount of compound to diffuse into the surrounding medium. In contrast, the disc diffusion method relies on the gradual release of the compound from an impregnated paper disc, which may limit diffusion, particularly for compounds exhibiting poor aqueous solubility or limited diffusion characteristics [24,25]. Despite these methodological differences, both assays consistently demonstrated the superior antifungal performance of ketoconazole compared with the investigated phytoconstituents.

Correlation Between In Silico and In Vitro Findings

An important outcome of the present investigation was the comparison between computational predictions and experimental observations. The molecular docking study indicated that quercetin possessed the strongest predicted interaction with CYP51 among the investigated phytoconstituents, followed by naringenin and resveratrol. This ranking was generally reflected in the experimental antifungal assays, where quercetin produced comparatively greater inhibition than the other natural compounds. Nevertheless, the overall antifungal activities of all three phytoconstituents remained substantially lower than that of ketoconazole despite their favourable docking scores. This finding illustrates an important limitation of molecular docking studies. Although docking provides useful information regarding the potential interaction between a ligand and its target protein, it cannot account for the numerous physicochemical and biological factors that influence activity under laboratory conditions. Factors such as aqueous solubility, diffusion within the agar medium, membrane permeability, intracellular accessibility, and chemical stability may all affect antifungal performance [17,24,27]. In addition, the lipid-dependent growth requirements of Malassezia furfur introduce experimental conditions that may further influence the distribution and availability of the investigated compounds during susceptibility testing [17,26]. Therefore, while molecular docking represents an effective screening approach, the present findings demonstrate that favourable binding affinity alone does not necessarily predict meaningful antifungal activity and should always be interpreted together with experimental evidence.

Overall Significance of the Study

The present investigation combined computational analysis, analytical characterization, and experimental antifungal evaluation to examine the potential of quercetin, naringenin, and resveratrol against Malassezia furfur. Molecular docking suggested that all three phytoconstituents could interact with the CYP51 active site, whereas UV–Visible and FT-IR analyses confirmed the identity and structural integrity of the investigated compounds before biological testing. Although quercetin consistently exhibited the most favourable overall performance among the investigated phytoconstituents, its antifungal activity remained considerably lower than that of ketoconazole under the experimental conditions employed. Collectively, the findings indicate that effective target binding represents only one aspect of antifungal drug performance and that successful biological activity depends on additional physicochemical and biological factors. The computational and experimental findings are discussed to provide an integrated interpretation of the antifungal potential of the investigated phytoconstituents. Attention is given to the relationship between the predicted and observed biological responses.

CONCLUSION:

The present investigation assessed the antifungal potential of quercetin, naringenin, and resveratrol against Malassezia furfur by combining computational analysis with experimental evaluation. Docking analysis demonstrated that all three phytoconstituents were able to occupy the CYP51 binding site, with quercetin showing the strongest predicted interaction among the investigated natural compounds, although its docking performance remained inferior to that of ketoconazole. UV–Visible and FT-IR analyses verified the identity and structural integrity of the selected phytoconstituents before biological assessment, ensuring the reliability of the subsequent findings. Despite encouraging computational results, the in vitro studies showed only modest antifungal activity for all three compounds, even at higher concentrations, whereas ketoconazole consistently produced markedly greater inhibition. These observations indicate that molecular docking alone cannot accurately predict antifungal efficacy, since biological performance is also governed by factors such as solubility, diffusion behaviour, membrane permeability, and compound availability at the site of action. Collectively, the findings suggest that although quercetin, naringenin, and resveratrol exhibit measurable interactions with CYP51, their antifungal activity against M. furfur

under the present experimental conditions remains limited. The study highlights the importance of complementing computational screening with laboratory-based validation and provides a basis for future investigations focused on structural optimization, formulation development, and advanced drug delivery strategies to improve the therapeutic potential of naturally occurring polyphenols.

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