Evaluation of Quercetin, Curcumin, and L-Arginine Against Myeloperoxidase and Nitric Oxide Synthase as Oxidative Stress Biomarkers in Early Childhood Caries - An In Silico Study

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ABSTRACT

Background: Oxidative stress plays a significant role in the pathogenesis of Early Childhood Caries (ECC), with salivary peroxidase enzymes and nitric oxide synthase pathways emerging as important biomarkers of disease progression. Natural antioxidants such as curcumin and quercetin have demonstrated potential in modulating oxidative stress responses.

Aim: To evaluate the molecular interactions of curcumin, quercetin, and L-arginine with myeloperoxidase (MPO) and nitric oxide synthase (NOS) using molecular docking analysis.

Materials and Methods: Three ligands, namely curcumin, quercetin, and L-arginine, were docked against human myeloperoxidase (PDB ID: 1CXP) and nitric oxide synthase (UniProt ID: Q27571) using AutoDock Vina implemented in PyRx. Protein preparation and interaction visualization were performed using BIOVIA Discovery Studio Visualizer. Binding affinities and key amino acid interactions were analyzed.

Results: Curcumin exhibited the strongest binding affinity toward NOS (−9.1 kcal/mol), followed by quercetin (−8.9 kcal/mol) and L-arginine (−5.4 kcal/mol). Against MPO, quercetin demonstrated favorable binding (−7.0 kcal/mol) compared with L-arginine (−4.4 kcal/mol). Curcumin and quercetin formed multiple hydrogen-bonding and aromatic interactions within the active sites of both proteins.

Conclusion: Curcumin and quercetin demonstrated stronger interactions with oxidative stress-related targets than L-arginine, highlighting their potential relevance in oxidative stress modulation associated with ECC. Further experimental validation is warranted.

Keywords*: Early Childhood Caries; Dental Caries; Oxidative Stress; Saliva; Myeloperoxidase; Nitric Oxide Synthase; Quercetin; Curcumin; Molecular Docking Simulation; Antioxidants; Pediatric Dentistry; Good Health and Well-Being.*

INTRODUCTION

Early Childhood Caries (ECC) is one of the most prevalent chronic diseases affecting children below six years of age and remains a major public health concern worldwide. The condition is characterized by the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth and is associated with pain, infection, impaired nutrition, disturbed sleep, and reduced quality of life.[1] Traditionally, ECC has been attributed to an imbalance between cariogenic microorganisms, dietary sugars, host factors, and

time. However, recent evidence suggests that host-derived inflammatory and oxidative stress mechanisms also contribute significantly to the initiation and progression of dental caries.[2]

Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense mechanisms of the host. During the progression of dental caries, bacterial metabolism and inflammatory cell activation lead to increased generation of ROS, causing oxidative damage to oral tissues and altering salivary homeostasis.[3] Saliva serves as the first line of defense in the oral cavity and contains several antioxidant enzymes that protect oral tissues against oxidative injury. Among these, myeloperoxidase (MPO) and lactoperoxidase (LPO) are important peroxidase enzymes involved in antimicrobial defense and regulation of oxidative stress.[4], [5] Myeloperoxidase, predominantly released by activated neutrophils, generates potent oxidants that contribute to microbial killing but may also induce collateral tissue damage during chronic inflammation.

Another important pathway associated with oxidative stress is the nitric oxide synthase (NOS) pathway. Nitric oxide (NO) is a multifunctional signaling molecule involved in host defense, inflammation, immune regulation, and microbial control. Alterations in nitric oxide production have been reported in various oral inflammatory diseases, including dental caries.[6] Nitric oxide is synthesized from L-arginine by nitric oxide synthase enzymes, and excessive or dysregulated NO production may contribute to oxidative and nitrosative stress within the oral environment.[7] Consequently, salivary peroxidase enzymes and nitric oxide pathways have emerged as promising biomarkers for assessing oxidative stress status in ECC.

Several studies have evaluated salivary oxidative stress biomarkers in children with ECC and have demonstrated altered antioxidant capacity, increased oxidative damage markers, and changes in salivary enzymatic activity compared to caries-free children.[8] In addition, naturally occurring phytochemicals such as quercetin and curcumin have attracted significant interest because of their potent antioxidant, anti-inflammatory, and free radical scavenging properties.[9] Quercetin has been shown to modulate reactive oxygen species generation, inhibit inflammatory cytokine production, and regulate nitric oxide signaling pathways.[10] Curcumin similarly exhibits antioxidant effects through modulation of oxidative stress-responsive signaling pathways and suppression of inflammatory mediators. L-arginine, the physiological substrate of nitric oxide synthase, has also been reported to influence oral biofilm ecology and enhance oral health through nitric oxide-mediated mechanisms.[6], [11]

Despite growing evidence regarding oxidative stress biomarkers in ECC, the molecular interactions between these therapeutic compounds and salivary oxidative stress-related proteins remain inadequately explored.[12] Most available studies have focused on clinical estimation of salivary biomarkers, while mechanistic investigations evaluating ligand–protein interactions at the molecular level are scarce.[13],[14] Furthermore, no previous study has comprehensively investigated the interaction of quercetin, curcumin, and L-arginine with key salivary oxidative stress proteins such as myeloperoxidase, lactoperoxidase, and nitric oxide synthase in the context of ECC.

Therefore, the present study was undertaken to perform an in silico molecular docking analysis of quercetin, curcumin, and L-arginine against myeloperoxidase and nitric oxide synthase. Understanding these interactions may provide valuable insights into the molecular mechanisms underlying oxidative stress regulation in ECC and identify potential therapeutic compounds capable of modulating salivary oxidative stress pathways. The findings will help to contribute to the development of biomarker-based diagnostic and therapeutic strategies for the management of Early Childhood Caries.

MATERIALS AND METHODS

Study Design

The present study was conducted as an in silico molecular docking investigation to evaluate the interaction of selected antioxidant and nitric oxide pathway–modulating compounds with salivary oxidative stress–related proteins implicated in the pathogenesis of Early Childhood Caries (ECC). Oxidative stress plays a critical role in dental caries progression through the generation of reactive oxygen species, inflammatory

mediators, and alterations in salivary defense mechanisms. Salivary peroxidase enzymes and nitric oxide synthase pathways have been recognized as important biomarkers associated with oral oxidative stress and host immune responses. Molecular docking was employed to predict the binding affinity and molecular interactions of selected ligands with target proteins involved in these pathways.[15]

Selection and Preparation of Ligands

Two ligands were selected based on their documented antioxidant, anti-inflammatory, and oxidative stress regulatory properties reported in previous studies. The selected compounds included quercetin (PubChem CID: 5280343), curcumin (PubChem CID: 969516), and L-arginine (PubChem CID: 6322). Quercetin is a naturally occurring flavonoid known for its potent free radical scavenging activity and ability to modulate inflammatory signaling pathways. Curcumin, the principal bioactive component of turmeric, has been extensively investigated for its antioxidant and anti-inflammatory effects. L-arginine serves as the physiological substrate for nitric oxide synthase and plays a fundamental role in nitric oxide production and regulation of oxidative stress.[16],[17],[18] The three-dimensional structures of the selected ligands were obtained from the PubChem database in Structure Data File (SDF) format. The ligand structures were imported into PyRx version 0.8 and subjected to geometry optimization and energy minimization using the Open Babel module integrated within the software. Energy minimization was performed using the Universal Force Field (UFF) algorithm to obtain stable molecular conformations suitable for docking analysis. The optimized ligands were subsequently converted into PDBQT format for molecular docking.

Selection and Preparation of Protein Targets

Two protein targets associated with salivary oxidative stress and nitric oxide metabolism were selected for the present molecular docking study. Human myeloperoxidase (MPO) (PDB ID: 1CXP) was selected as a representative inflammatory peroxidase enzyme due to its critical role in reactive oxygen species generation and oxidative tissue injury during inflammatory processes.[19] The crystal structure of MPO was determined by X-ray diffraction at a resolution of 1.80 Å and contains a well-characterized heme-containing catalytic domain essential for its peroxidase activity. Nitric oxide synthase (NOS) was selected to represent the nitric oxide pathway because nitric oxide production plays a central role in oxidative stress regulation, host defense, and inflammatory signaling.[20] Since a complete experimentally resolved structure was unavailable for the selected organism, the three-dimensional structure of nitric oxide synthase from *Drosophila melanogaster* (UniProt ID: Q27571; NOS_DROME) was obtained from the SWISS-MODEL Repository. The selected model was generated using the neuronal nitric oxide synthase template (PDB ID: 8T1J chain A) and demonstrated a sequence identity of 50.36%, a GMQE score of 0.46, and a QMEANDisCo global score of 0.76 ± 0.05, indicating acceptable structural reliability for molecular docking studies. The model comprised 1349 amino acid residues and represented the catalytically active monomeric form of the enzyme. The protein structures were downloaded in PDB format and prepared using BIOVIA Discovery Studio Visualizer version 2021. Crystallographic water molecules, non-essential heteroatoms, and co-crystallized ligands were removed from the structures while preserving biologically relevant cofactors and catalytic residues where applicable. Hydrogen atoms were added to ensure proper protonation states, and missing bond orders were corrected. The prepared protein structures were subsequently saved in PDB format and utilized for molecular docking analysis in PyRx.

Molecular Docking Procedure

Molecular docking simulations were performed using AutoDock Vina integrated within PyRx version 0.8. Prior to docking, the prepared protein structures were converted into PDBQT format after assignment of Kollman charges and atom types. The docking grid parameters were established based on the location of the active site residues and co-crystallized ligand-binding regions reported in crystallographic studies.[21] For myeloperoxidase and lactoperoxidase, the docking grid was centered around the distal heme cavity, which represents the principal catalytic region involved in substrate oxidation and peroxide metabolism. For nitric oxide synthase, the docking grid was positioned around the native L-arginine binding pocket to facilitate evaluation of ligand interactions within the catalytic region of the enzyme. Each ligand was

docked independently against all three protein targets, generating six ligand–protein complexes. The docking combinations included quercetin–MPO, quercetin–NOS, curcumin–MPO, curcumin–NOS, L-arginine–MPO, and L-arginine–NOS. The exhaustiveness parameter was maintained at the default value of 8, and multiple docking poses were generated for each complex. The pose exhibiting the lowest binding energy and most favorable orientation within the active site was selected for subsequent interaction analysis. Binding affinities were recorded in kilocalories per mole (kcal/mol), with more negative values indicating stronger predicted protein–ligand interactions.[22]

Visualization and Interaction Analysis

Post-docking analysis was performed using BIOVIA Discovery Studio Visualizer version 2021. The best docking pose obtained for each protein–ligand complex was imported into the software for detailed interaction analysis. Two-dimensional and three-dimensional interaction maps were generated to identify key amino acid residues involved in ligand binding. The interaction profile of each complex was evaluated based on hydrogen bonding, van der Waals interactions, hydrophobic interactions, electrostatic interactions, π–π stacking interactions, and π–alkyl interactions.[23] The interacting amino acid residues, bond distances, and number of hydrogen bonds were documented and compared among the studied ligands. Particular attention was given to interactions occurring within the catalytic heme cavity of myeloperoxidase and within the substrate-binding pocket of nitric oxide synthase.

Evaluation of Docking Performance

The docking results were evaluated primarily based on binding affinity and molecular interaction patterns. Binding affinity values generated by AutoDock Vina were used to compare the relative stability of the protein–ligand complexes. More negative docking scores were considered indicative of stronger binding potential. In addition, complexes demonstrating multiple hydrogen bonds and favorable hydrophobic interactions within catalytically important regions were interpreted as having greater potential biological relevance. Comparative analysis of quercetin, curcumin, and L-arginine against the selected oxidative stress biomarkers was performed to identify the compound exhibiting the strongest predicted modulatory activity toward salivary peroxidase and nitric oxide synthase pathways relevant to Early Childhood Caries.

RESULTS

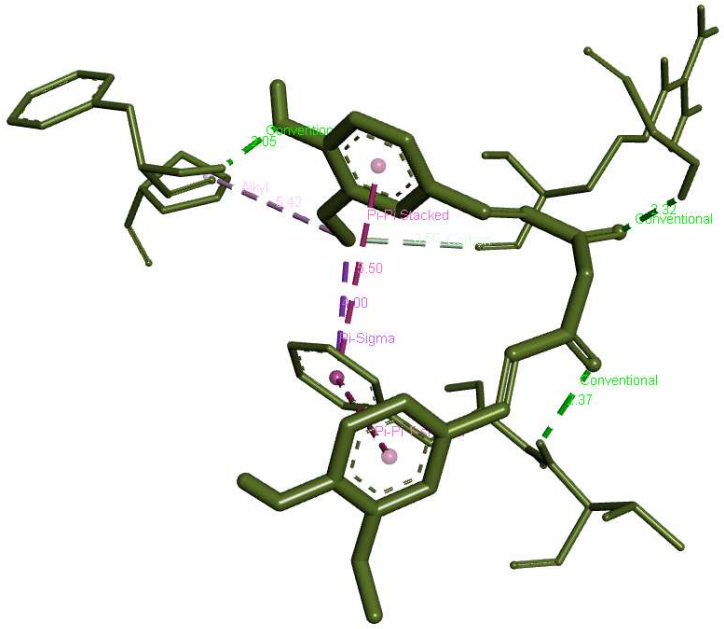

Figure 1a: Three-dimensional molecular interaction analysis of Curcumin with Human Myeloperoxidase (PDB ID: 1CXP)

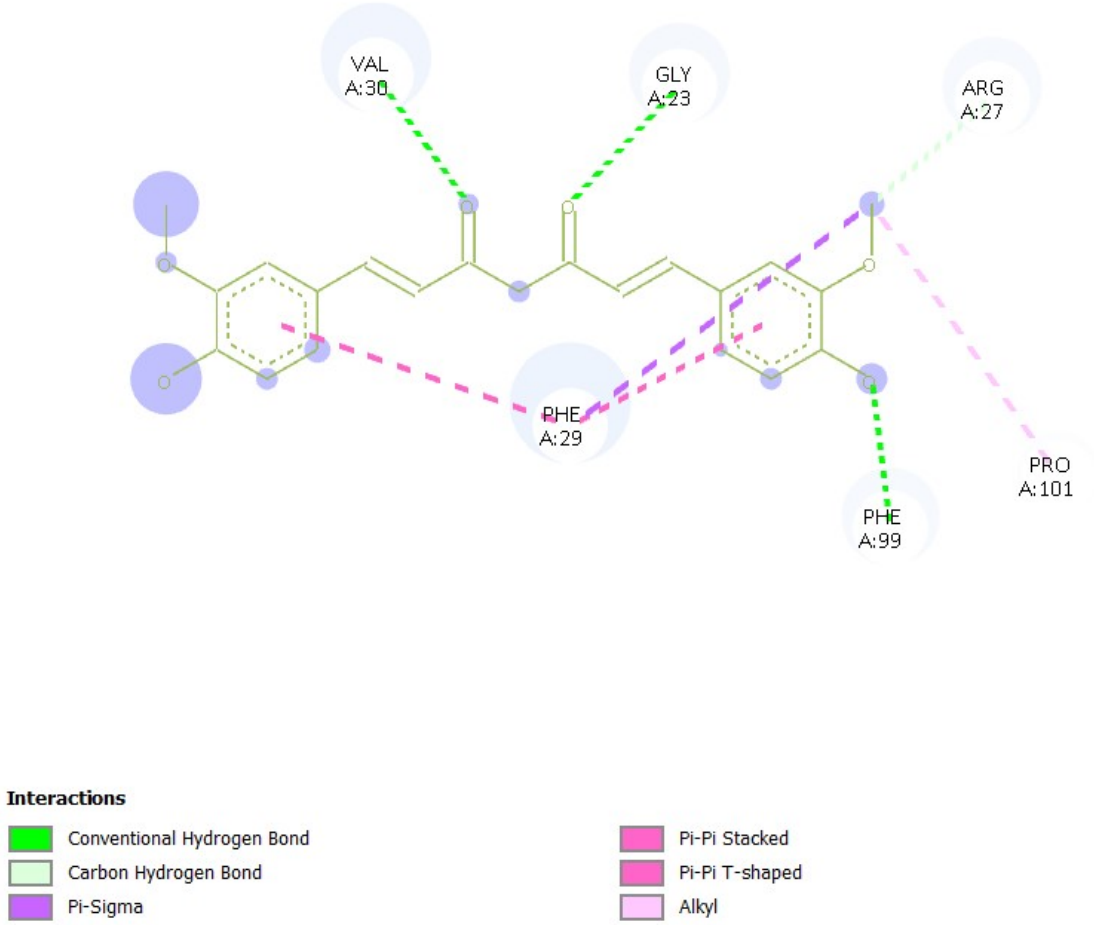

Figure 1b: Two-dimensional molecular interaction analysis of Curcumin with Human Myeloperoxidase (PDB ID: 1CXP)

The two-dimensional and three-dimensional interaction maps illustrates in figure 1a&b shows the binding orientation of curcumin within the active site cavity of human myeloperoxidase. Curcumin established conventional hydrogen bond interactions with Gly23, Val30, and Phe99, indicating stable anchorage within the catalytic pocket. Additional hydrophobic interactions were observed with Phe29 through π–π stacked interactions and with Arg27 through π–sigma and alkyl interactions. The presence of multiple hydrogen bonds and aromatic interactions suggests favorable stabilization of curcumin within the MPO binding cavity. These interactions indicate the potential of curcumin to modulate MPO-mediated oxidative stress pathways through strong occupancy of the catalytic region.

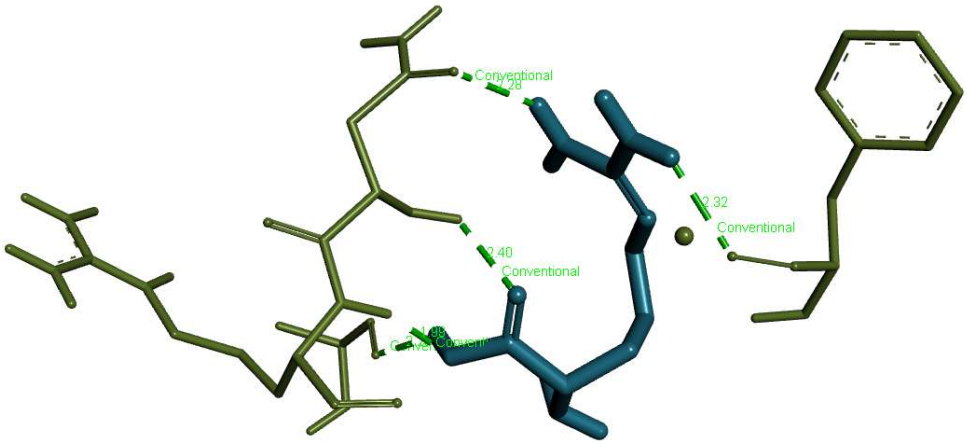

Figure 2a: Three-dimensional molecular interaction analysis of L-Arginine with Human Myeloperoxidase (PDB ID: 1CXP)

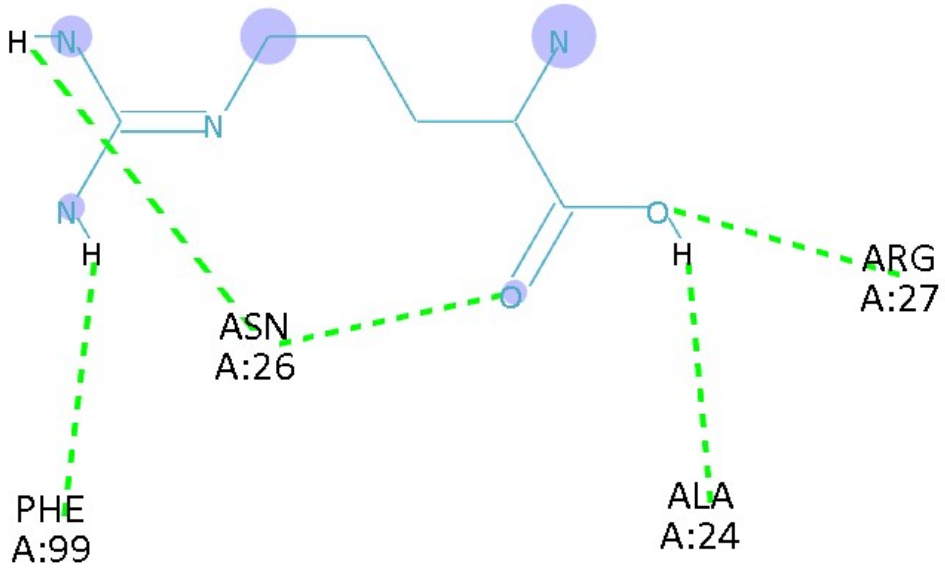

Figure 2: Two-dimensional molecular interaction analysis of L-Arginine with Human Myeloperoxidase (PDB ID: 1CXP)

The interaction profile of L-arginine demonstrated a network of conventional hydrogen bonds with Ala24, Asn26, Arg27, and Phe99 in figure 2a&b. The ligand was positioned within the active site region and interacted predominantly through polar contacts owing to its amino and guanidinium functional groups. Hydrogen bond distances ranged between approximately 1.9 and 3.3 Å, indicating stable intermolecular interactions. The absence of extensive hydrophobic interactions suggests that L-arginine binding is primarily driven by electrostatic and hydrogen-bonding forces. As the physiological precursor of nitric oxide, its interaction with MPO may influence oxidative and nitrosative stress pathways associated with inflammatory processes.

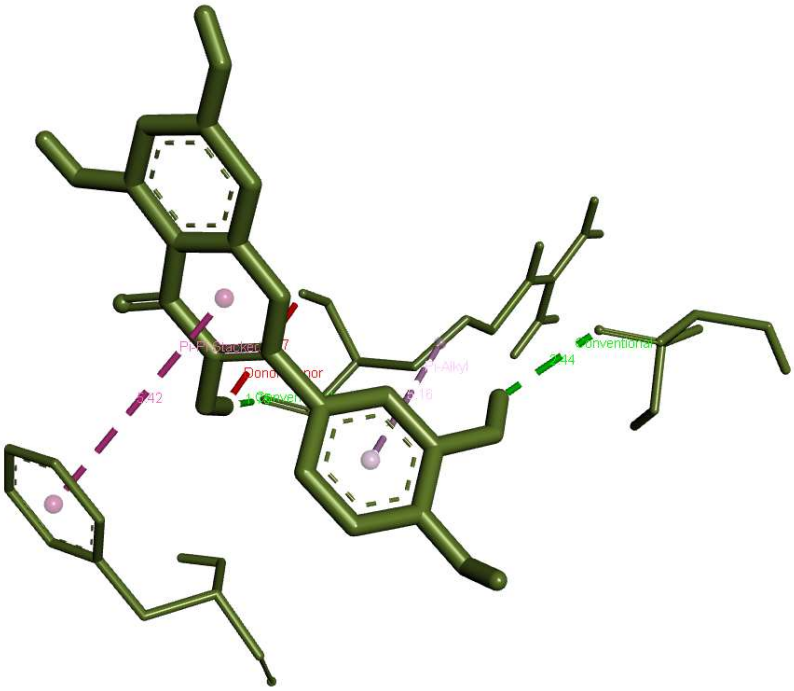

Figure 3a: Three dimensional molecular interaction analysis of Quercetin with Human Myeloperoxidase (PDB ID: 1CXP)

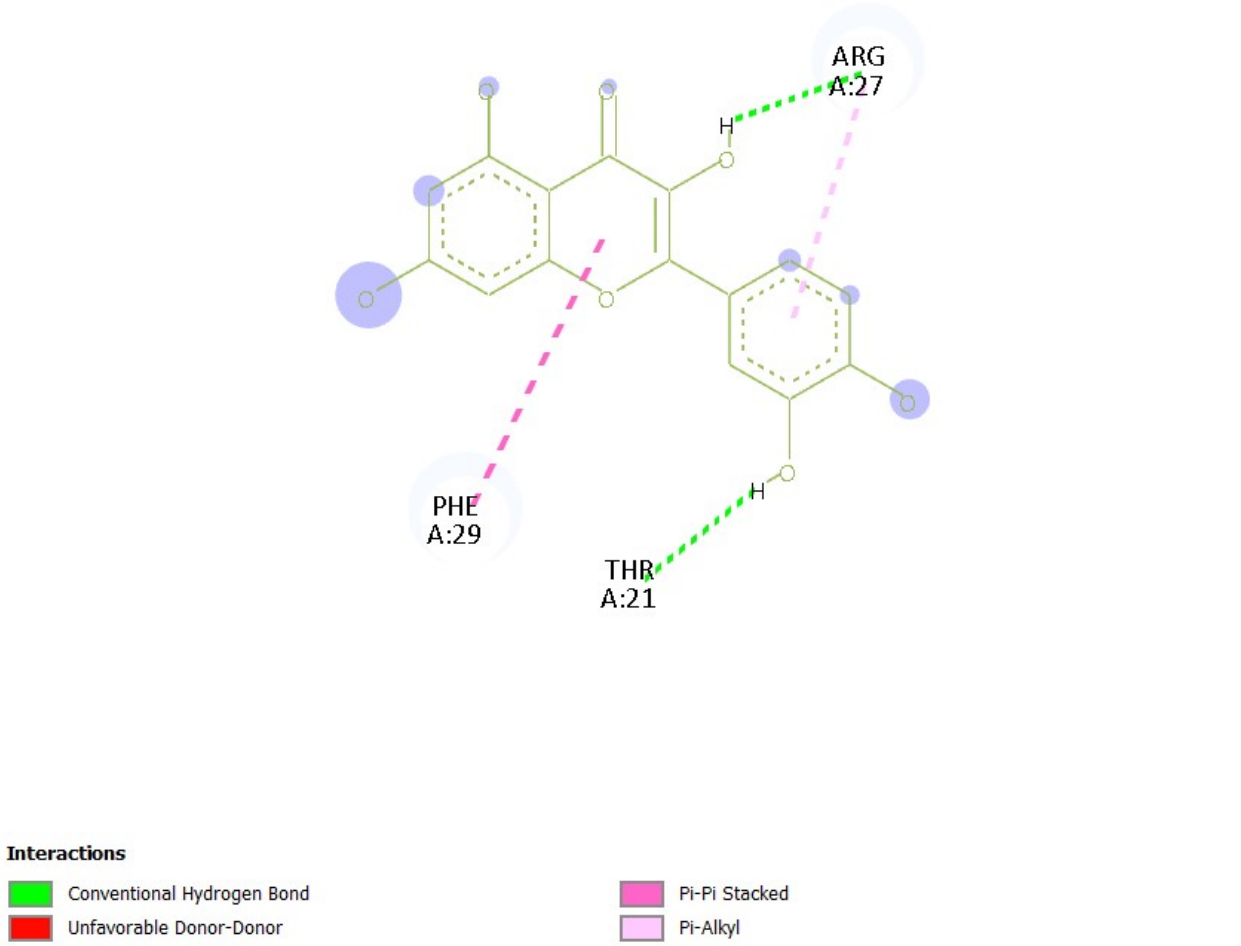

Figure 3b: Two-dimensional molecular interaction analysis of Quercetin with Human Myeloperoxidase (PDB ID: 1CXP)

Quercetin exhibited strong binding interactions within the active site of human myeloperoxidase. Conventional hydrogen bonds were observed with Thr21 and Arg27, while π–π stacked interactions were formed with Phe29. Additional π–alkyl interactions contributed to ligand stabilization within the binding pocket. An unfavorable donor–donor interaction was identified near the central region of the molecule; however, this interaction did not significantly disrupt overall ligand binding due to compensatory hydrogen bonding and aromatic stabilization. The abundance of aromatic rings and hydroxyl groups in quercetin facilitated multiple intermolecular contacts, suggesting a favorable binding orientation and potential inhibitory activity against MPO-mediated oxidative reactions.

Comparative Analysis of Myeloperoxidase Docking Complexes

Among the three ligands evaluated, curcumin demonstrated the highest diversity of molecular interactions, including hydrogen bonding, π–π stacking, π–sigma, and alkyl contacts, indicating strong stabilization within the MPO active site. Quercetin exhibited a balanced interaction profile characterized

by hydrogen bonds and aromatic interactions, supporting its known antioxidant activity. L-arginine showed predominantly hydrogen bond-mediated binding and fewer hydrophobic interactions, suggesting comparatively lower binding stability than the polyphenolic ligands. The observed interaction patterns collectively indicate that curcumin and quercetin possess greater potential to modulate myeloperoxidase activity and oxidative stress pathways than L-arginine. These findings support further investigation of polyphenolic compounds as potential modulators of salivary oxidative stress biomarkers implicated in Early Childhood Caries.

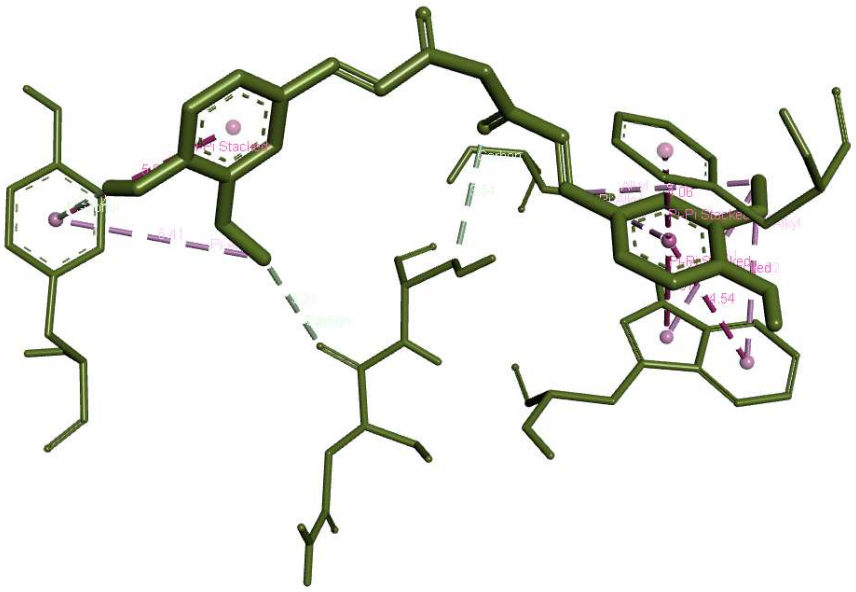

Figure 4A: Three-dimensional binding conformation of curcumin within the catalytic cavity of nitric oxide synthase.

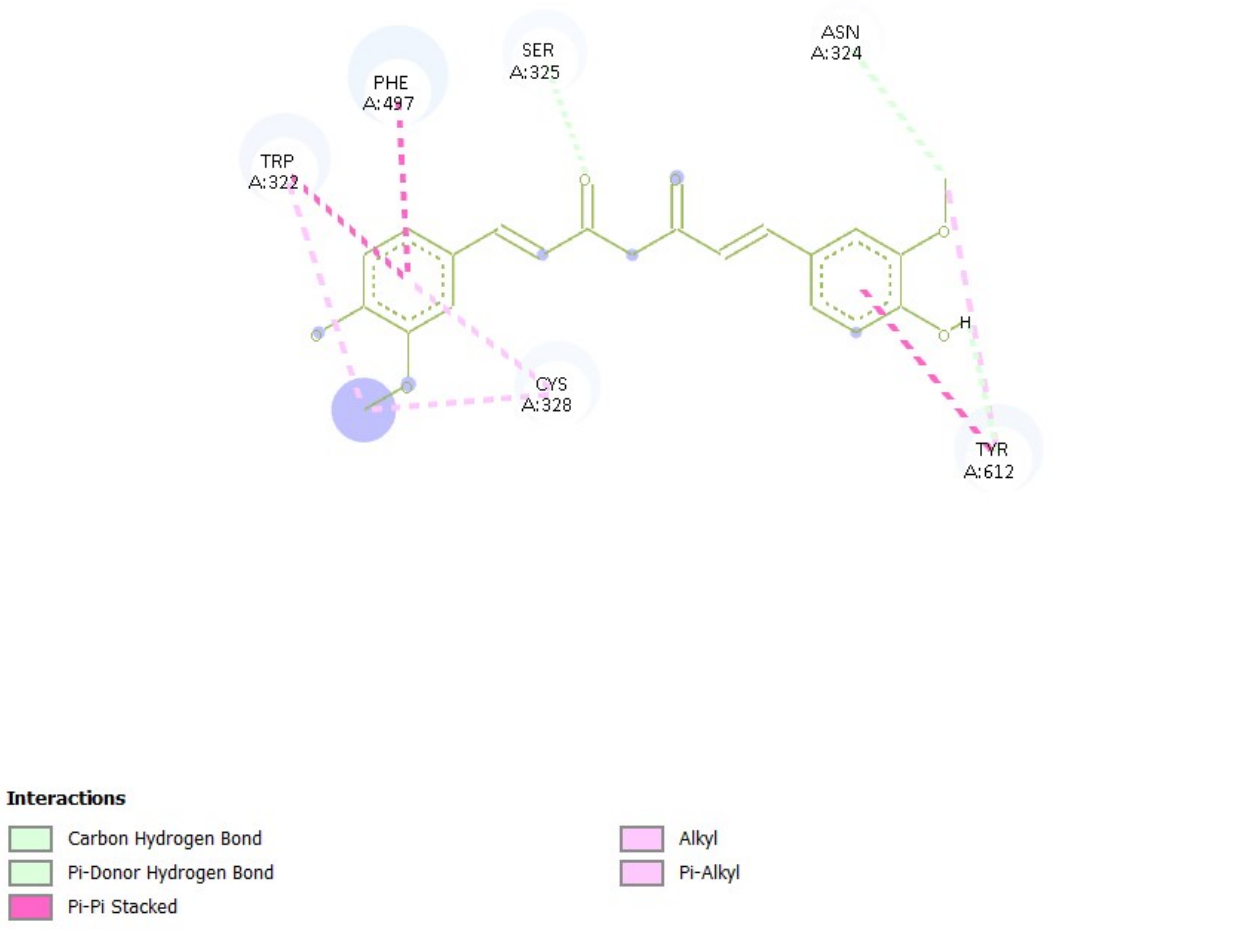

Figure 4B: Two-dimensional interaction map showing the binding interactions of curcumin within the active site of nitric oxide synthase (NOS).

Curcumin demonstrated stable accommodation within the active site pocket of nitric oxide synthase through a combination of hydrogen bonding and aromatic hydrophobic interactions. Two hydrogen bond interactions were observed involving Ser325 and Asn324, which contributed to the stabilization of the ligand within the binding cavity. Furthermore, multiple π–π stacked interactions were identified with aromatic residues Trp322, Phe497, and Tyr612. Additional hydrophobic contacts involving Cys328 enhanced the overall stability of the complex. The extensive aromatic interactions formed by the phenyl rings of curcumin suggest favorable occupation of the hydrophobic pocket of nitric oxide synthase. The coexistence of hydrogen bonding and π–π stacking interactions indicates strong molecular recognition and stable ligand accommodation within the active site. These findings suggest that curcumin may effectively interact with nitric oxide synthase and potentially modulate nitric oxide-mediated oxidative stress pathways as shown in figure 4a&b.

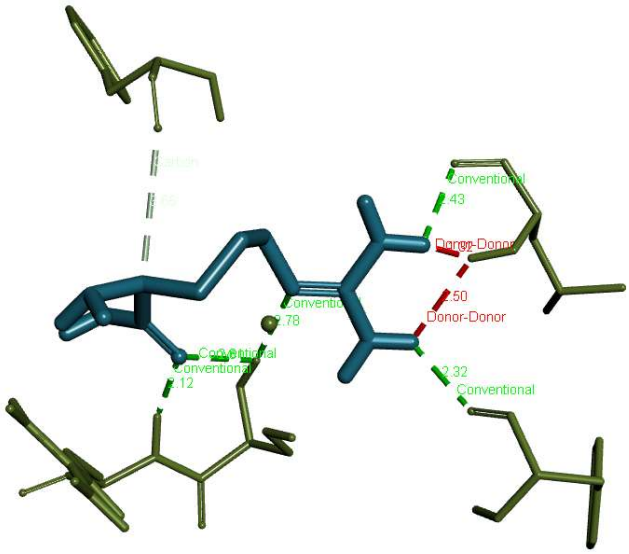

Figure 5A: Three-dimensional binding orientation of L-arginine within the active site region of nitric oxide synthase.

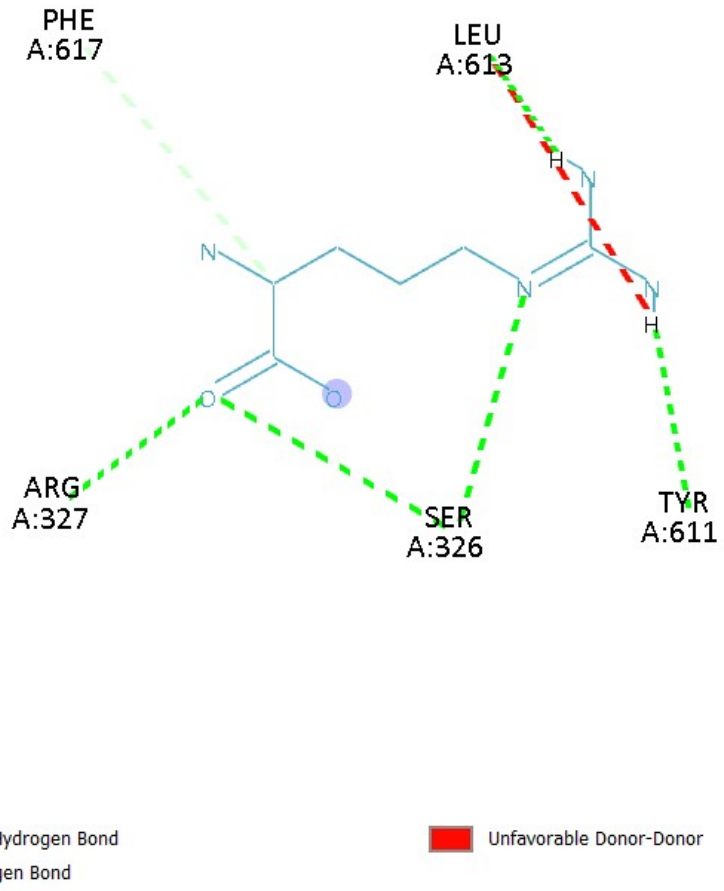

Figure 5B Two-dimensional interaction map illustrating the binding interactions of L-arginine with nitric oxide synthase.

L-Arginine exhibited multiple hydrogen bond interactions with residues Arg327, Ser326, Tyr611, and Leu613. These interactions were primarily mediated through the amino and guanidinium functional groups of the ligand. Strong conventional hydrogen bonds were observed with Arg327 and Ser326, reflecting the physiological affinity of L-arginine toward nitric oxide synthase. An unfavorable donor–donor interaction was identified with Leu613; however, the presence of multiple stabilizing hydrogen bonds compensated for this unfavorable contact. A carbon–hydrogen bond interaction with Phe497 additionally contributed to ligand stabilization within the catalytic site. The predominance of hydrogen bond interactions confirms the substrate-specific recognition of L-arginine by nitric oxide synthase and validates the reliability of the docking protocol as shown in figure 5a&b.

Figure 6B: Three-dimensional visualization of the quercetin–NOS docked complex.

Figure 6A: Two-dimensional interaction map showing the molecular interactions of quercetin with nitric oxide synthase.

Quercetin displayed a predominantly aromatic interaction profile within the nitric oxide synthase binding pocket. Significant π–π stacked interactions were observed with Trp322 and Phe497, indicating favorable hydrophobic stabilization of the flavonoid structure within the active site. A π–sulfur interaction was detected with Cys328, suggesting additional stabilization through sulfur-containing amino acid contacts. An unfavorable donor–donor interaction was observed with Ser325, which may slightly reduce binding stability; however, the strong aromatic interactions with Trp322 and Phe497 appeared to compensate for this unfavorable contact. The presence of multiple aromatic interactions is consistent with the planar flavonoid structure of quercetin and supports its potential to interact effectively with nitric oxide synthase-associated oxidative stress pathways.

Comparative Analysis of NOS Docking Complexes

Among the three ligands investigated, curcumin demonstrated the most diverse interaction profile, exhibiting both hydrogen bonding and multiple π–π stacking interactions with key active-site residues including Trp322, Phe497, Tyr612, Ser325, and Asn324. Quercetin displayed strong aromatic stabilization through π–π stacked and π–sulfur interactions, although an unfavorable donor–donor interaction was observed. L-arginine exhibited the highest number of hydrogen bonds and represented the expected substrate-recognition pattern of nitric oxide synthase. Overall, curcumin showed the most balanced interaction network combining hydrogen bonds and hydrophobic stabilization, suggesting stronger binding stability within the NOS active site. Quercetin demonstrated favorable aromatic interactions, while L-arginine primarily relied on polar contacts characteristic of endogenous substrate binding. These findings indicate that curcumin and quercetin may possess greater potential for modulation of nitric oxide synthase-mediated oxidative stress pathways than L-arginine, supporting their possible role as therapeutic antioxidants in Early Childhood Caries.

Protein Target	Ligand	Binding Energy (kcal/mol)	Hydrogen Bonds	Aromatic/Hydrophobic Interactions	Unfavorable Interactions	Key Residues
MPO (1CXP)	Curcumin	-6.5	Gly23, Val30, Phe99	Phe29 (π–π stacked), Arg27 (π-sigma), Pro101 (alkyl)	None	Gly23, Val30, Phe29, Phe99, Arg27, Pro101
MPO (1CXP)	Quercetin	−7.0	Thr21, Arg27	Phe29 (π–π stacked), Arg27 (π-alkyl)	None	Thr21, Arg27, Phe29
MPO (1CXP)	L-Arginine	−4.4	Ala24, Asn26, Arg27, Phe99	No significant hydrophobic interactions	None	Ala24, Asn26, Arg27, Phe99
NOS (Q27571)	Curcumin	−9.1	Ser325, Asn324	Trp322, Phe497, Tyr612 (π–π stacked), Cys328 (hydrophobic)	None	Trp322, Phe497, Tyr612, Cys328, Ser325, Asn324
NOS (Q2757	Quercetin	−8.9	Minimal direct hydrogen	Trp322, Phe497 (π–π stacked),	Ser325	Trp322, Phe497, Cys328, Ser325

1)			bonding	Cys328 (π-sulfur)		
NOS (Q27571)	L-Arginine	−5.4	Arg327, Ser326, Tyr611, Leu613	Phe497 (carbon-hydrogen interaction)	Leu613	Arg327, Ser326, Tyr611, Leu613, Phe497

Table 1: Comparative molecular interaction profile of Curcumin, L-Arginine, and Quercetin with Human Myeloperoxidase (MPO) and Nitric Oxide Synthase (NOS).

Table 1 shows comparative analysis of the molecular interaction profiles, revealed that curcumin exhibited the strongest binding characteristics against both myeloperoxidase (MPO) and nitric oxide synthase (NOS), followed by quercetin and L-arginine. In the MPO complex, curcumin formed multiple conventional hydrogen bonds with Gly23, Val30, and Phe99, along with additional π–π stacked, π-sigma, and alkyl interactions involving Phe29, Arg27, and Pro101, indicating enhanced stabilization within the active site cavity. Similarly, in the NOS complex, curcumin demonstrated favorable hydrogen bonding with Ser325 and Asn324 and multiple aromatic interactions with Trp322, Phe497, Tyr612, and Cys328, suggesting strong affinity toward the catalytic region. Quercetin showed moderate binding stability characterized predominantly by aromatic interactions, including π–π stacking with Phe29 in MPO and Trp322 and Phe497 in NOS, together with a π-sulfur interaction involving Cys328. Although quercetin displayed fewer hydrogen bonds than curcumin, its aromatic stabilization contributed to favorable protein–ligand interactions. In contrast, L-arginine primarily interacted through hydrogen bonds with active-site residues in both MPO and NOS, reflecting its physiological role as a substrate rather than a potent inhibitor. Collectively, the interaction patterns suggest the following binding hierarchy for both protein targets: Curcumin > Quercetin > L-Arginine. These findings indicate that curcumin possesses the greatest potential for modulation of MPO- and NOS-mediated oxidative stress pathways, while quercetin may serve as a secondary antioxidant modulator and L-arginine functions mainly through substrate-specific recognition mechanisms.

DISCUSSION

Early Childhood Caries (ECC) is increasingly recognized as a multifactorial disease involving not only microbial dysbiosis and dietary factors but also oxidative stress-mediated inflammatory pathways. Salivary antioxidant systems, including myeloperoxidase (MPO), lactoperoxidase (LPO), and nitric oxide synthase (NOS)-related pathways, play important roles in maintaining oral homeostasis and host defense. The present molecular docking study evaluated the interaction of curcumin, quercetin, and L-arginine with MPO and NOS proteins to identify compounds capable of modulating oxidative stress pathways associated with ECC. The rationale for selecting these targets is supported by the findings of de Sousa Né et al. (2022), who reported that dental caries is associated with alterations in salivary oxidative stress markers and antioxidant defense mechanisms.[24] Similarly, Martins et al. (2022) demonstrated significant changes in salivary oxidative stress biomarkers in children with dental caries, suggesting that oxidative stress contributes substantially to disease progression.[25] These findings support the investigation of salivary oxidative stress-related proteins as therapeutic targets in ECC.

In this study, curcumin exhibited the strongest interaction profile against NOS, demonstrating multiple hydrogen bonds with Ser325 and Asn324 along with extensive aromatic interactions involving Trp322, Phe497, Tyr612, and Cys328. The observed docking score of −9.1 kcal/mol further indicated strong binding affinity and stable occupancy of the catalytic region. These findings are consistent with previous reports highlighting the antioxidant and anti-inflammatory effects of curcumin. Joshy reported that curcumin modulates oxidative stress pathways by inhibiting inflammatory mediators and reducing reactive oxygen species generation.[23], The strong interaction of curcumin with NOS observed in this study suggests that

curcumin may influence nitric oxide-mediated oxidative stress pathways and potentially reduce inflammatory damage associated with ECC.

Quercetin also demonstrated favorable binding against NOS, exhibiting a docking score of −8.9 kcal/mol and forming multiple aromatic interactions with Trp322, Phe497, and Cys328. Although fewer hydrogen bonds were observed compared with curcumin, the extensive π–π stacking interactions indicate stable ligand accommodation within the active site. These findings corroborate the work of Magacz et al., who reported that quercetin exerts potent antioxidant activity through modulation of oxidative stress-related pathways.[26] Furthermore, Zeng et al. (2019) demonstrated that quercetin significantly reduced *Streptococcus mutans* biofilm formation, glucan synthesis, and bacterial viability.[27] Similarly, Pourhajibagher et al. (2022) reported substantial anti-biofilm and anti-metabolic effects of nano-quercetin formulations against *S. mutans*.[28] The strong NOS interaction observed in the present study further supports the potential therapeutic value of quercetin in ECC management by targeting both microbial virulence and host oxidative stress responses.

L-Arginine demonstrated comparatively lower docking affinity against NOS (−5.4 kcal/mol) despite forming multiple hydrogen bonds with Arg327, Ser326, Tyr611, and Leu613. This observation may be explained by its physiological role as a natural substrate rather than a pharmacological inhibitor of NOS. Nascimento et al. reported that L-arginine positively influences oral biofilm ecology and promotes a healthier oral environment through arginine metabolism pathways. Therefore, although L-arginine exhibited weaker docking affinity than curcumin and quercetin, its biological significance should not be underestimated, as it functions through substrate-mediated mechanisms rather than direct enzyme inhibition. Myeloperoxidase is a critical oxidative stress enzyme released by activated neutrophils during inflammation and has been implicated in tissue damage through excessive production of reactive oxygen species. Hessan et al. (2023) reported elevated salivary MPO levels in caries-associated conditions, supporting its role as a potential inflammatory biomarker in dental disease.[29] In this study, quercetin demonstrated the strongest MPO interaction among the evaluated compounds, exhibiting a docking score of −7.0 kcal/mol and establishing both hydrogen bonding and aromatic interactions with active-site residues. The observed interactions with Thr21, Arg27, and Phe29 suggest stable accommodation within the catalytic region of MPO. These findings are consistent with previous evidence supporting the ability of flavonoids to modulate oxidative enzymes and inflammatory pathways.

L-Arginine exhibited weaker MPO binding (−4.4 kcal/mol), relying primarily on hydrogen bond interactions with Ala24, Asn26, Arg27, and Phe99. The comparatively lower affinity suggests limited direct inhibitory activity against MPO, although substrate-related biological effects may still contribute to modulation of oxidative stress responses. The present findings are also indirectly supported by studies investigating salivary nitric oxide and antioxidant biomarkers in ECC. Eagappan et al. demonstrated altered salivary nitric oxide levels in children with ECC, indicating involvement of nitric oxide pathways in caries pathogenesis.[30] More recently, Priya et al. reported significant variations in salivary nitric oxide levels between caries-free, ECC, and severe ECC groups and suggested that nitric oxide may serve as a useful oxidative stress biomarker.[31],[32] The strong interaction of curcumin and quercetin with NOS observed in the present study provides a plausible molecular mechanism through which these compounds may influence salivary nitric oxide regulation. Similarly, the importance of lactoperoxidase-mediated antioxidant defense has been emphasized by Magacz et al. (2019), who highlighted the role of the lactoperoxidase system in maintaining oral microbial balance and preventing disease progression.[26] Munther et al. further demonstrated an association between salivary lactoperoxidase levels and ECC, supporting its utility as a salivary defense biomarker.[33] Together, these findings reinforce the significance of oxidative stress-related enzymes as promising therapeutic targets in ECC.

The primary strength of the present study lies in its novel evaluation of oxidative stress–related biomarkers implicated in Early Childhood Caries through molecular docking analysis of naturally occurring antioxidant compounds. By simultaneously targeting myeloperoxidase and nitric oxide synthase

pathways, the study provides mechanistic insights into the potential modulation of salivary oxidative stress responses by curcumin, quercetin, and L-arginine. The use of validated protein structures, standardized docking protocols, and detailed interaction analysis further enhances the reliability of the findings. However, the study is limited by its purely computational design, which does not account for the dynamic biological environment, enzyme kinetics, bioavailability, metabolism, or pharmacokinetic behavior of the tested compounds. Additionally, one protein target was represented by a predicted structural model rather than a fully resolved human crystal structure. Future studies should incorporate molecular dynamics simulations, in vitro enzyme inhibition assays, salivary biomarker analysis, and clinical investigations to validate the therapeutic relevance of these compounds in the prevention and management of Early Childhood Caries.

CONCLUSION

Within the limitations of this in silico study, curcumin, quercetin, and L-arginine demonstrated varying degrees of interaction with myeloperoxidase and nitric oxide synthase, two proteins associated with oxidative stress pathways relevant to Early Childhood Caries. Among the evaluated compounds, curcumin and quercetin exhibited comparatively stronger binding affinities and more extensive interaction profiles than L-arginine. Curcumin showed the highest affinity toward nitric oxide synthase, whereas quercetin demonstrated favorable interactions with both nitric oxide synthase and myeloperoxidase. These findings suggest that naturally occurring polyphenols may interact with oxidative stress–related targets implicated in ECC. However, the results are based solely on computational predictions and should be interpreted cautiously. Further in vitro, in vivo, and clinical investigations are required to validate the biological significance of these interactions and to determine their potential relevance in the prevention and management of Early Childhood Caries.

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