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PREPARATION AND CHARACTERIZATION OF CURCUMIN AND PIPERINE NANOPARTICLES AND IN SILICO ANALYSIS OF THEIR INTERACTION AGAINST GLYCOSYLTRANSFERASE OF *STREPTOCOCCUS MUTANS* AND *LACTOBACILLUS RHAMNOSUS*.

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ABSTRACT:

Aim: To prepare and characterize nanocurcumin and nanopiperine and investigate their insilico interaction against glycosyltransferase (GTFs) of *Streptococcus mutans* and *Lactobacillus rhamnosus*.

Materials and methodology: Curcumin and Piperine nanoparticles were prepared via sonication and characterized using dynamic light scattering (DLS), UV-Vis spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy.

For docking, the molecular structures of the targets and ligands were downloaded from RCSB PDB and PubChem database respectively. The preparation of the target and ligands were done using AutoDock 4.2.6 software. The molecular docking study was then carried out using AutoDock vina and interpreted using BIOVIA Discovery Studio software.

Results: DLS revealed an average diameter of 111.2 nm and 110.1 nm for nanocurcumin and nanopiperine, respectively. UV–visible spectra of nanocurcumin and nanopiperine showed characteristic peaks at 425 nm and 350 nm respectively. FTIR spectrum of curcumin and piperine showed peaks corresponding to the functional groups present in the phytocompounds. Docking results demonstrated the efficacy of curcumin and piperine against the glycosyltransferases of *S. mutans* and *L. rhamnosus*.

Conclusion: Based on the results, we can conclude that, sonication method proves to be an effective and simple approach for nanoparticle preparation of curcumin and piperine. And these phytochemicals are effective against GTFs of *S. mutans* and *L. rhamnosus*.

Key words: Nanocurcumin, Nanopiperine, Nanophytochemicals, Sonication, Molecular docking, Glycosyltransferase

INTRODUCTION

Caries is one of the most prevalent microbial diseases that affect people worldwide. It was a well-established fact that endogenous gram-positive bacteria *Streptococcus mutans* (*S. mutans*) is the chief cariogenic agent.(1) Later literatures show the parallel role of *lactobacilli* on the progression and pathogenesis of dental caries.(2) The cariogenic nature of both the *S. mutans* and the *Lactobacillus sp.* stems from its capacity to form biofilms, facilitated by a specific set of membrane-bound enzymes known as Glycosyltransferases (GTFs). GTFs are responsible for the development of virulent dental plaque by adsorbing on to enamel and synthesizing glucans in-situ hence providing sites for avid colonization by the microorganisms.(3) Schwendicke *et al* in 2014, demonstrated that the combined biofilm model of *Streptococcus mutans* and *Lactobacillus rhamnosus* exhibited multifold demineralization of both enamel and

dentin.[4] The matured biofilm entities have tolerance mechanisms more pronounced than the planktonic counterparts which hinder the treatment options and lead to persistent bacterial infections.[5]

Contemporary approaches in anticariogenic therapy should be focused on disrupting or preventing the formation of these multi-species biofilms.[6] Various synthetic agents such as Tween 80, Quaternary Ammonium Compounds, Triton X- 100, non-ionic surfactants, rhamnolipids are used for biofilm disruption. With the advancements of synthetic agents, there is a parallel known increase in bacterial resistance as well.[5] The increase in antibiotic-resistant strains and side effects caused by synthetic agents has prompted researchers to look for herbal alternatives. In recent years, a developing interest has been observed in evaluating different herbal extracts for their antimicrobial properties against microorganisms causing dental caries.[7] There are literature evidences that herbal agents such as *Aloe barbadensis* (Aloe vera), *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Matricaria recutitia* (Chamomile), *Citrus limonum* (Lemon), *Morinda citrifolia* (Noni), *Propolis* (Bee glue), *Psidium guajava* (Guava), *Salvadora persica* (Miswak), *Syzygium aromaticum* (Clove), *Melaleuca alternifolia* (Tea tree), *Curcuma longa* (Turmeric), *Glycyrrhiza glabra* (Licorice) can be used as alternative antimicrobial agents because of their antimicrobial, anti-inflammatory and immune-modulating properties.[8-10]

Nano formulations of such phytocompounds are regarded as a substitute for antibiotics in combating multidrug-resistant and biofilm-associated infections. Nanoparticles present distinct advantages over their bulk counterparts due to their nano size and high surface area to volume ratio. These benefits encompass high reactivity, improved solubility and the potential for functionalization with various drugs and bioactive molecules. Additionally, antimicrobial nanoparticles demonstrate superior ability to infiltrate biofilms, exhibit potency at reduced doses, and offer promise in addressing the growing concern of antibiotic resistance.[11]

Curcumin and piperine are the most commonly used Indian herbal agents, widely known for their therapeutic uses. Therapeutic potential of curcumin and piperine includes antimicrobial, anti-inflammatory, antioxidant and anticarcinogenic properties.[12,13] The nano formulations of these phytocompounds could enhance their response to specific molecular targets and improve their pharmacological activity.[11] Flora *et al* in 2013 stated that, nano curcumin has multifold effectiveness than native curcumin and was found to have a higher intracellular absorption capacity compared to normal curcumin.[14] Curcumin encapsulated in

nanoparticles of polylactic acid and dextran sulfate resulted in the photoinactivation of methicillin-resistant Staphylococcus aureus (MRSA), *S. mutans*, and *C. albicans*.[15] Study by Ahrari *et al* (2023) demonstrated that Photodynamic therapy with nanomicelle curcumin 3g/L could disrupt *L. casei* biofilms.[16] Gopalakrishna *et al.* (2023) provided evidence for the efficacy of piperine-loaded in situ gels in combating plaque, gingival and periodontal diseases.[17]

Literature describes various methodologies for nanoparticle preparation, both with and without the use of carriers.[18] However, despite the availability of numerous methods, there remains a critical need for a simpler yet effective approach for preparing nanoparticles of these phytochemicals. This study aims to prepare and characterize nanocurcumin and nanopiperine and use molecular docking to assess the interaction of these phytocompounds with glycosyltransferase of *Streptococcus mutans* and *Lactobacillus rhamnosus*.

MATERIALS AND METHODOLOGY:

I. PREPARATION OF NANOPARTICLES:

Preparation of Curcumin Nanoparticles:

This study employed sonication method for the nanoparticle preparation, a technique suitable for drugs with limited water solubility. 240 mg of curcumin (Sigma Aldrich, Merck, India) was dissolved in 1 ml of ethanol, and 29 ml of hexane was then added to this mixture to attain a concentration of 8 mg/ml, while maintaining a hexane to ethanol volume ratio of 30:1. Following 30 minutes of sonication, the mixture was stirred with a magnetic stirrer to maintain suspension homogeneity and then heated to 80°C for 15 minutes. The resulting dry curcumin nanocrystals were collected and sealed for subsequent use.

Preparation of Piperine Nanoparticles:

Similarly, 240 mg of Piperine (Sigma Aldrich, Merck, India) was dissolved in 30 ml of ethanol. This mixture was subjected to 30 minutes of sonication, then stirred with a magnetic stirrer to maintain suspension homogeneity and heated to 80°C for 15 minutes. The resulting dry piperine nanocrystals were collected and sealed for subsequent use.

II. CHARACTERISATION OF THE NANOPARTICLES:

1. Dynamic light scattering:

The solutions of the nanoparticles were prepared by taking 1 mg each of the nanocurcumin and nanopiperine powders separately in 10 mL of ethanol. The solutions were filtered by spin-filtering. Approximately 30 µL of each sample was taken for the cuvette and

evaluated under DLS S90 series. The Dyna-Pro software showed an interface with two sides, the left measures the count number and the right side reveals the calculated data.

2. FTIR (Fourier Transform Infrared) spectroscopy:

Curcumin and piperine nanoparticle specimens were placed in between the probe and the platform, following which the FTIR spectra was recorded in the range of 400–4000 cm^{-1} using a Nicolet FTIR spectrophotometer. The bands obtained corresponded to the vibrations of the functional groups present in the respective nanoparticles

3. Ultraviolet–visible (UV-Vis) spectroscopy:

The optical property of synthesized nanocurcumin and nanopiperine was analysed by taking the absorption spectra between 200 and 800 nm and measuring maximum absorbance using a UV-visible spectrophotometer.

III. MOLECULAR DOCKING:

The molecular level interactions of curcumin and piperine with glycosyltransferase of *Streptococcus mutans* and *Lactobacillus rhamnosus* were carried out using AutoDock Vina.

Preparation of protein:

The selected target protein was the crystal structure of Glycosyltransferase of *Streptococcus mutans* (PDB id: 8UF5) (figure 1a) and *Lactobacillus rhamnosus* (Uniprot id: K8QH77) (figure 1b). The PDB (Protein Data Bank) format of the target protein was retrieved from RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) database and visualized and optimized using BIOVIA Discovery Studio software. The optimization of the target protein was performed by deleting the heteroatoms and water molecules. The polar hydrogens were added and active site was determined. The attributes of xyz were -12.386761, 65.429943, 8.229364 and 2.593427, 0.471387, -1.271290 for *Streptococcus mutans* and *Lactobacillus rhamnosus* respectively. Target protein was converted from PDB to PDBQT **(**Protein Data Bank, Partial Charge (Q) & Atom Type (T)**)** format using AutoDock 4.2.6 software.

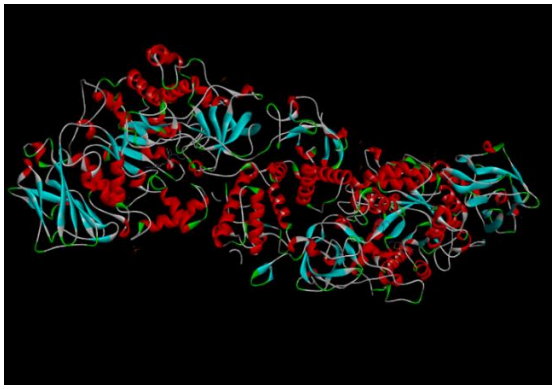

Fig 1a: Glycosyltransferase of *Streptococcus mutans*

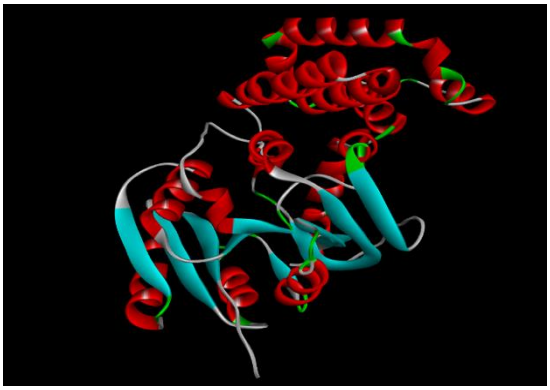

Fig 1b: Glycosyltransferase of *Lactobacillus rhamnosus*

Figure 1: Structure of target protein (figure 1a,1b)

Preparation of Ligands:

The 3-dimensional molecular structure of the phytocompounds, curcumin (PubChem id: 969516) (Figure 2a) and piperine (PubChem id: 638024) (Figure 2b) were downloaded from the PubChem website in 3D SDF (Structure-Data Files) format and visualized using BIOVIA Discovery Studio software and converted from SDF to PDB format. Preparation of ligand was performed using AutoDock 4.2.6 software.

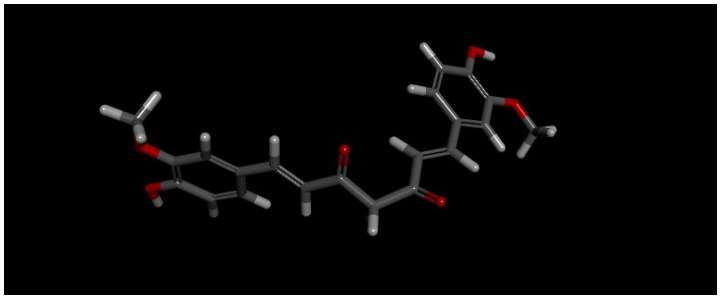

Figure 2a: Structure of Curcumin

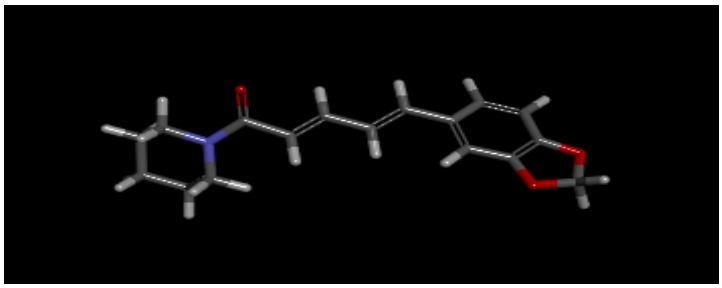

Figure 2b: Structure of Piperine

Molecular Docking study

The optimized ligands were docked with Glycosyltransferase of *Streptococcus mutans* (PDB id: 8UF5) *and Lactobacillus rhamnosus* (Uniprot id: K8QH77) using AutoDock vina software. To perform molecular docking, conf file was prepared and subjected to molecular docking. The molecular docking analysis was done based on the Lamarckian genetic algorithm with a maximum of 2.5 million energy evaluations. Molecular Docking was performed with AutoDock vina using command prompt and the topmost conformation of the docked complex was interpreted using BIOVIA Discovery Studio software.

RESULTS:

Dynamic light scattering of the dispersion of nanocurcumin and nanopiperine in ethanol showed an average hydrodynamic diameter of 111.2 nm and 110.1 nm respectively. (Fig 3a, 3b)

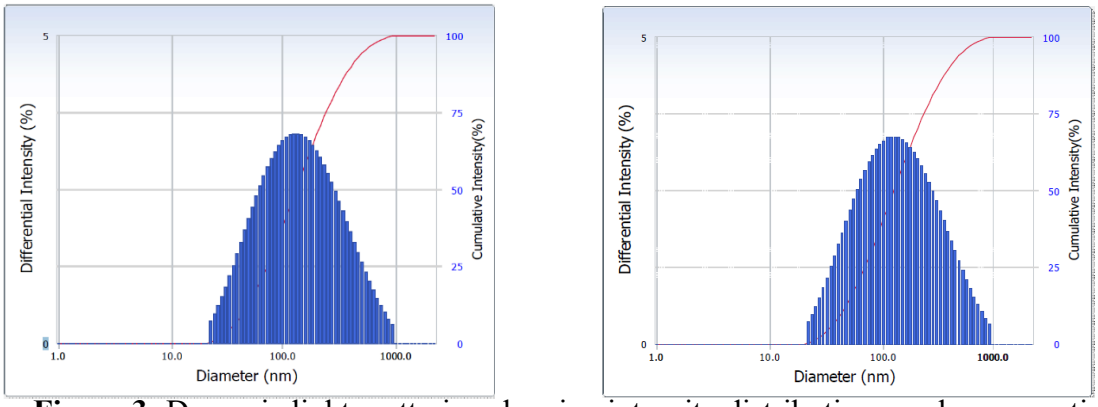

Figure 3: Dynamic light scattering showing intensity distribution graph representing the average hydrodynamic size of (**a**) nanocurcumin; (**b**) nanopiperine particles

In *Fourier transform infrared spectroscopy*, the IR spectrum of curcumin demonstrated stretching vibrations due to phenolic hydroxyl groups at 3411 cm-1, peak at 1633 cm^{-1} typically corresponds to the stretching vibration of the carbonyl (C=O) group, peak at 1424 cm^{-1} may correspond to the bending vibration of the aromatic C-H bonds, the peak at 1330 cm^{-1} could be attributed to the stretching vibration of the C-O bond, the peak at 1121 cm^{-1} might correspond to the stretching vibration of the C-O-C ether linkage, the peak at 1052 cm^{-1} could be associated with the stretching vibration of C-OH groups. (Fig 4a)

The IR spectrum of piperine demonstrated stretching vibrations due to phenolic hydroxyl groups at 3399 cm-1, peak at 1627 cm-1 suggestive of C=O stretching, peak at 1417

cm^{-1} could indicate C-H bending vibrations, possibly from aliphatic groups, the peak at 1334 cm^{-1} could be attributed to the stretching vibration of the C-O bond, the peak at 1124 cm^{-1} might correspond to the stretching vibration of the C-O/ C-C, the peak at 1032 cm^{-1} could be associated with the stretching vibration of C-H groups. (Fig 4b)

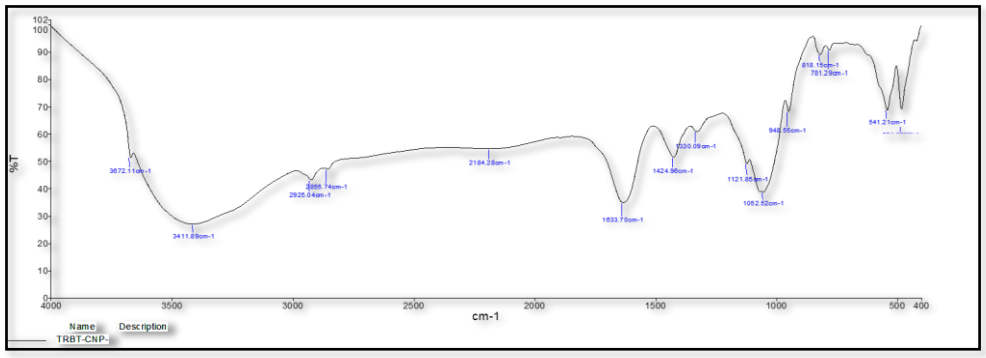

Figure 4a

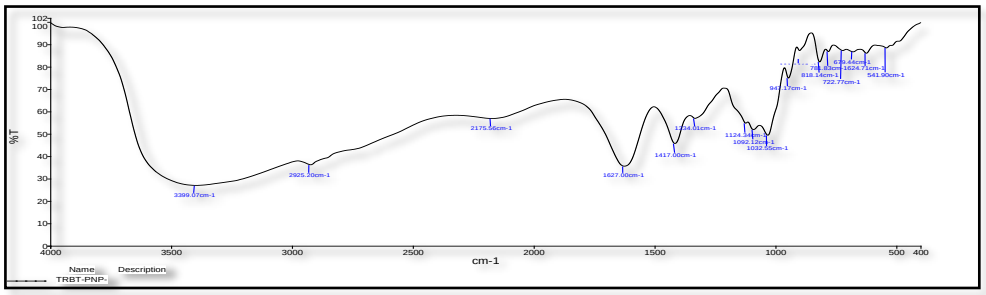

Figure 4b

Figure 4: FTIR spectrum of (a) Nanocurcumin (b) Nanopiperine

UV–visible spectroscopy: The results showed a characteristic peak of nanocurcumin at 425 nm, while the nanopiperine showed a peak at 350 nm. (Fig 5a,5b)

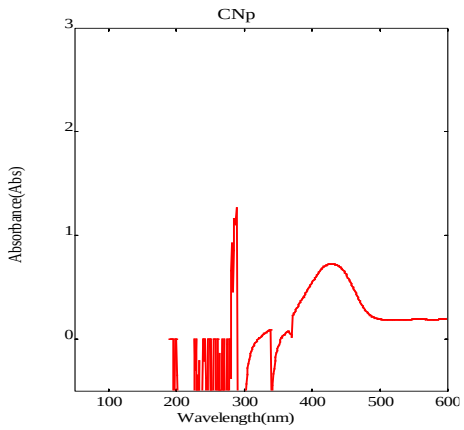

Fig 5a

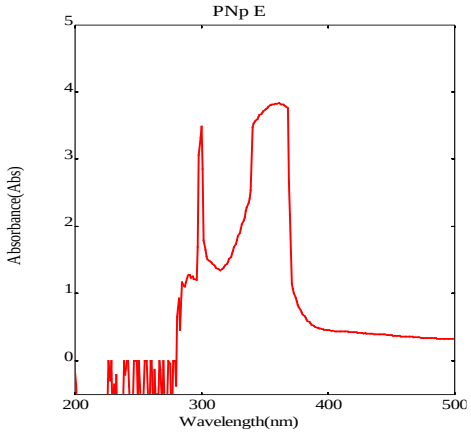

Fig 5b

Figure 5: UV–visible spectrum of (**a**) nanocurcumin; (**b**) nanopiperine.

In molecular docking the phytocompounds, curcumin and piperine were individually docked against the GTFs of *Streptococcus mutans* and *Lactobacillus rhamnosus* and the topmost conformation of the docked complex was interpreted.

1. Curcumin against Glycosyltransferase of *Streptococcus mutans* showed binding affinity of -7.10 Kcal/mole with 5 hydrogen bonds. The amino acid residues that were involved in the H bond were ASN(A) 294, SER(B) 346, VAL(B) 582, THR(A) 312, GLU(B) 349. There was also presence of 2 carbon hydrogen bonds and 1 hydrophobic pi sigma bond.

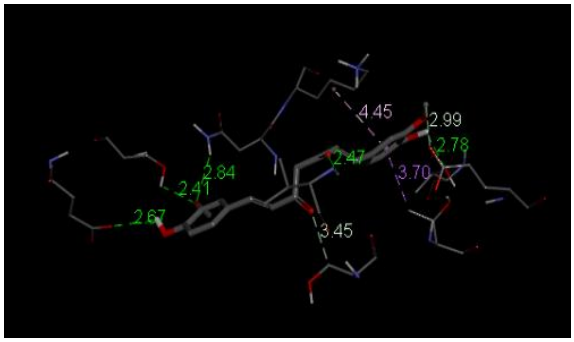

Fig 6a

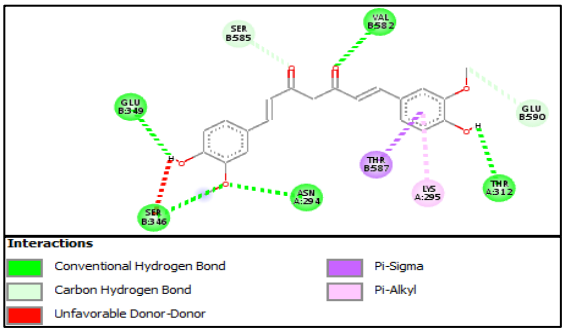

Fig 6b

Figure 6: Molecular docked complex of Curcumin and Glycosyltransferase of *Streptococcus mutans* (**a**) 3 D structure; (**b**) 2 D structure

2. Curcumin against Glycosyltransferase of *Lactobacillus rhamnosus* showed binding affinity of -7.8 Kcal/mole with 4 hydrogen bonds. The amino acid residues that were involved in the H bond were TYR(A) 210, SER (A) 216, PRO (A) 13, SER (A) 94. There was also presence of 1 Electrostatic pi anion bond and 1 pi donor hydrogen bond.

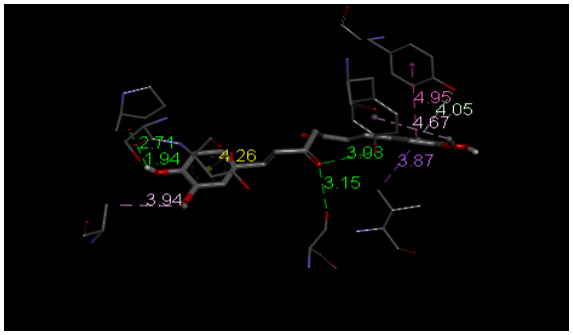

Fig 7a

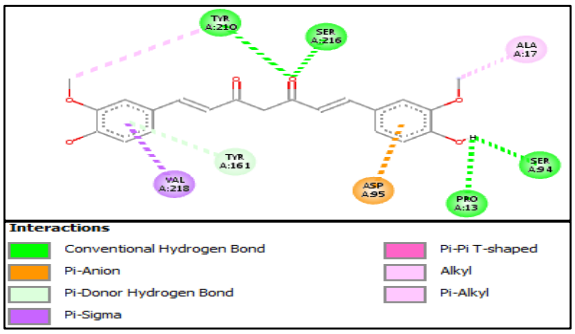

Fig 7b

Figure 7: Molecular docked complex of Curcumin and Glycosyltransferase of *Lactobacillus rhamnosus* (**a**) 3 D structure; (**b**) 2 D structure

3. Piperine against Glycosyltransferase of *Streptococcus mutans* showed binding affinity of -7.3 Kcal/mole with 2 hydrogen bonds. The amino acid residues that were involved in the H bond were ASN (B) 578, ASN (B) 578. There was also presence of 1 carbon hydrogen bond, 1 hydrophobic alkyl bond and 1 hydrophobic pi alkyl bond.

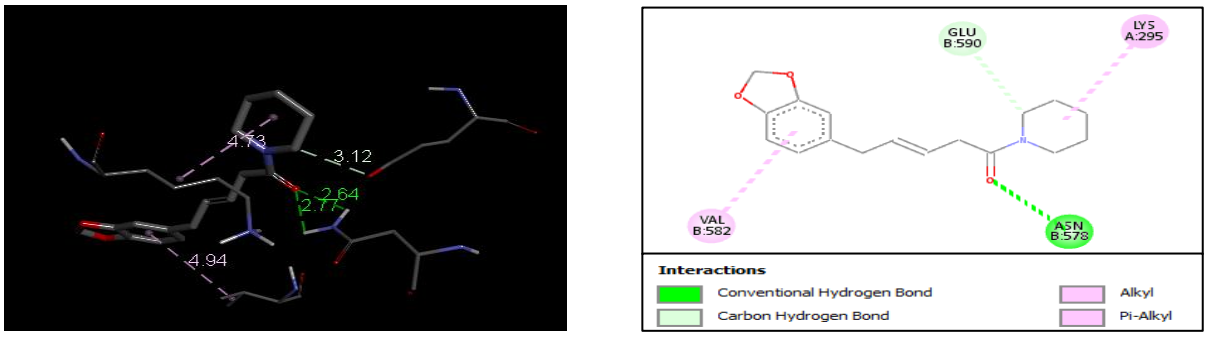

Fig 8a **Fig 8b**

Figure 8: Molecular docked complex of Piperine and Glycosyltransferase of *Streptococcus mutans* (**a**) 3 D structure; (**b**) 2 D structure

4. Piperine against glycosyltransferase of *Lactobacillus rhamnosus* showed binding affinity of -7.6 Kcal/mole with 1 hydrogen bond. The amino acid residues that were involved in the H bond were SER (A) 94. There was also presence of 1 electrostatic pi anion bond.

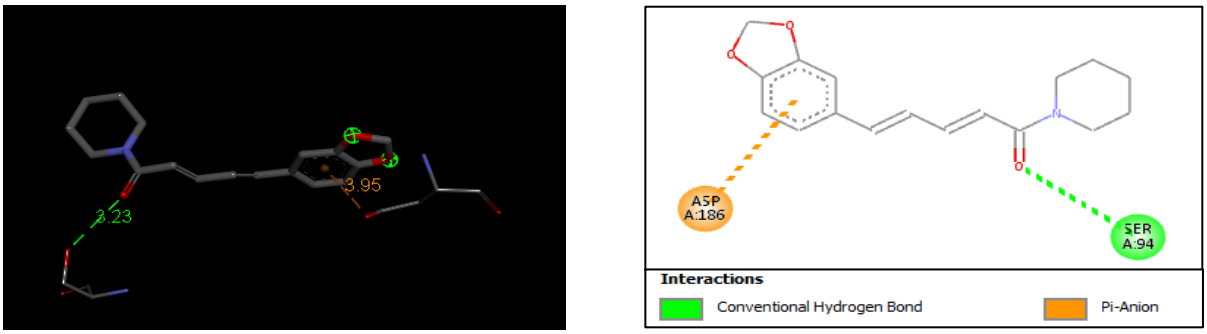

Fig 9a **Fig 9b**

Figure 9: Molecular docked complex of Piperine and Glycosyltransferase of *Lactobacillus rhamnosus* (**a**) 3 D structure; (**b**) 2 D structure

Table 1: Molecular docking results showing the binding energy, the bond formation and interactions with specific amino acid residues for each docked complex.

S.No	Binding affinity	Bonds	Amino acid Residues
8UF5_Curcumin	-7.10 Kcal/mole	5 Hydrogen bonds	ASN (A) 294-2.84 Å SER (B) 346- 2.41 Å VAL (B) 582 – 2.47Å THR (A) 312- 2.78 Å GLU (B) 349- 2.67 Å
		2 Carbon hydrogen bonds	SER (B) 585- 3.45 Å GLU (B) 590- 2.99 Å
		1 Hydrophobic pi sigma bond	THR (B) 587- 3.69 Å LYS (A) 295- 4.45 Å
LR_Curcumin	-7.8 Kcal/mole	4 Hydrogen bonds	TYR(A) 210 - 3.08 Å SER (A) 216- 3.15 Å PRO (A) 13- 2.71 Å SER (A) 94- 1.94 Å
		1 Electrostatic pi anion bond	ASP (A) 95 – 4.26 Å
		1 Pi donor hydrogen bond	TYR (A) 161- 4.05 Å
		1 hydrophobic pi-sigma bond	VAL (A) 218- 3.86 Å
		1 pi-pi T shaped hydrophobic bond	TYR (A) 161-4.95 Å
		1 Hydrophobic alkyl bond	ALA (A) 17- 3.96 Å
		1 Hydrophobic pi-alkyl bond	TYR (A) 210- 4.67 Å
8UF5_Piperine	-7.3 Kcal/mole	2 Hydrogen bonds	ASN (B) 578-2.64 Å ASN (B) 578- 2.77 Å
		1 Carbon hydrogen bond	GLU (B) 590 – 3.12Å
		1 Hydrophobic alkyl bond	LYS (A) 295- 4.73 Å
		1 Hydrophobic pi alkyl bond	VAL (B) 582- 4.94 Å
LR_Piperine	-7.6 Kcal/mole	1 Hydrogen bond	SER (A) 94-3.23 Å
		1 Electrostatic pi anion bond	ASP (A) 186 – 3.95Å

DISCUSSION:

S. mutans is one of the primary pathogens of dental caries that adheres to the tooth surface via biofilms (sucrose dependent mode of adherence) and cell wall surface proteins (sucrose independent mode of adherence) thus producing pathogenic acid metabolites by fermentation of carbohydrates and resulting in demineralization of tooth tissues.[19] The enzyme GTF of *S. mutans* is responsible for the production of extracellular polysaccharides, particularly glucans which contributes to the cariogenicity of the biofilms.[20] Similarly, in the clinical isolates of *L. rhamnosus* the presence of family 2 - glycosyltransferase was predicted to alter exopolysaccharides composition which could influence pathogenicity of the biofilm.[21] Hence, the suppression of GTF activity and the subsequent polysaccharide synthesis could potentially reduce the virulence of cariogenic biofilms, offering an alternative way to combat dental caries. To date, numerous GTF inhibitors have been identified in natural products, which represent a significant yet largely untapped reservoir of such inhibitors.[20]

Curcumin, with its array of biological benefits encompassing antioxidant, anticancer, anti-inflammatory, and antimicrobial properties, also demonstrates antibiofilm activity against a variety of microorganisms. Research indicates its effectiveness in combating biofilms formed by *Pseudomonas aeruginosa, Acinetobacter baumannii, Listeria monocytogenes, Enterococcus faecalis,* mixed species biofilms of *Candida albicans* and *Staphylococcus aureus*, among others. These findings indicate curcumin's potential as a valuable agent in addressing biofilm-related infections. However, challenges persist in the clinical application of curcumin due to issues such as low solubility, poor absorption, and rapid metabolism, all of which contributes to its limited bioavailability.[12]

Strategies to increase the bioavailability of curcumin includes co-administration with adjuvants or by nano formulations.[22] Curcumin has been co-administered with various natural substances such as piperine, quercetin, resveratrol, and silibinin which reduced the metabolism of curcumin, thus increasing its bioavailability. Among them, piperine is the most commonly available and widely used substance. Piperine is an alkaloid substance found in black pepper (*Piper nigrum*) and is proven to be a potent inhibitor of drug metabolism thus altering the bioavailability of a large number of drugs.[23] This has been proved in a study done by *Shoba et al*. (1998) who examined the serum levels of curcumin both with and without simultaneous oral administration of piperine. A remarkable 2000% increase in curcumin's bioavailability

within a 45 minute time frame was observed when taken orally alongside piperine, compared to curcumin alone.(24)

Literature search reveals no previous studies evaluated the effect of curcumin and piperine against GTFs of *Streptococcus mutans* and *Lactobacillus rhamnosus*. Therefore, a molecular docking investigation was conducted to assess the antibiofilm efficacy of curcumin and piperine against the biofilm-forming proteins of *Streptococcus mutans* and *Lactobacillus rhamnosus*.

In recent times, molecular docking has become integral to the field of in-silico drug development. This technique involves the precise prediction of how a drug interacts with a protein at the molecular level. Through molecular docking, researchers can gain insight into how drugs, including phytocompounds, behave within the binding site of a target protein, providing valuable understanding of the underlying biochemical processes involved.(25) The assessment of binding affinity in the docking experiment was based on two main criteria: the E-value (representing binding energy) and the number of hydrogen bonds established between the target molecule and the ligand. Involvement in electrostatic hydrogen bonding, both as a donor and an acceptor, is acknowledged as a vital interaction especially for noncovalent binders, alongside ionic and Van der Waals interactions. A lower E-value indicates a stronger and more stable binding interaction. Moreover, the presence of hydrogen bonding further enhances the binding affinity, with the highest negative energy values signifying strong binding affinity to specific residues.(26)

In the present study, both curcumin and piperine exhibited strong binding affinities with the target protein, as indicated by their negative binding energy values (-7.10 & -7.8 kcal/mol for curcumin and -7.3 & -7.6 kcal/mol for piperine). Curcumin forms multiple hydrogen bonds and hydrophobic interactions, indicating a diverse interaction profile. Piperine also forms hydrogen bonds and hydrophobic interactions, although with slightly different residues compared to curcumin.

Curcumin forms multiple hydrogen bonds with residues such as ASN (Asparagine), SER (Serine), THR (Threonine), VAL(Valine), PRO (Proline), TYR(Tyrosine) and GLU (Glutamic acid), indicating a versatile binding mode, whereas piperine forms hydrogen bonds with residues, such as ASN(Asparagine) and SER(Serine) as shown in table 1, figure 6-9. Curcumin and piperine both engage in hydrophobic interactions with the target protein,

typically mediated by non-polar amino acid residues which are essential for enhancing the stability of the ligand-protein complex. The strong binding affinities and diverse interaction profiles of curcumin and piperine prove their potential utility as lead compounds for drug development against the glycosyltransferases of *S. mutans* and *L. rhamnosus.*

In this study, curcumin and piperine were synthesized in nano formulations using sonication to increase its bioavailability and solubility. Nano particles formulations are suitable for controlled and targeted drug delivery systems. The nanoparticles have a relatively larger surface area, and it increases the rate of degradation and aqueous solubility, which leads to enrichment of the bioavailability of drugs. Nevertheless, a large surface area enhances a drug response to a specific molecular target and improves its pharmacological activity.[27] An array of techniques are available for synthesis of nanocurcumin which includes nanoprecipitation, single emulsion, microemulsion, spray drying, emulsion polymerization, solvent evaporation, antisolvent precipitation, ultra-sonication, coacervation technique, ionic gelation, wet milling, solid dispersion, thin-film hydration, and Fessi method. These techniques for preparation of curcumin nanoparticles, each with its own advantages and limitations, have been extensively explored in the literature.[18]

In this study, we opted to synthesize curcumin nanoparticles without employing any nanocarriers, utilizing the sonication method. This approach is commonly utilized for drugs with limited water solubility. Zhang *et al.* and Hettiarachchi *et al* have previously demonstrated successfully, the synthesis of curcumin nanoparticles using ultrasonication without the use of nanocarriers. The size of the obtained nanoparticles was in the range of 80 ± 20 nm through the sonication process.[28,29]

In the present study, we adapted a partially modified and a simple method for synthesizing curcumin and piperine nanoparticles, followed by characterization using dynamic light scattering, Ultraviolet–visible spectroscopy, and Fourier-transform infrared spectroscopy. The dynamic light scattering analysis revealed that both the nanocurcumin and nanopiperine aqueous dispersions exhibited an average hydrodynamic diameter of 111.2 nm and 110.1 nm, respectively. The UV–visible spectrum indicated the successful preparation of nanocurcumin and nanopiperine by showing a characteristic peak at 425 nm and 350 nm respectively. This confirms their nano-scale dimensions and affirms the successful formulation of curcumin and piperine in nanosized structures. In the FTIR spectrum of curcumin and piperine, the peaks

corresponded to the functional groups and bond environments present in the phytocompounds. The FTIR results of curcumin and piperine were similar to the results of Ghosh A *et al* and Dara M A *et al*. respectively.[30,31]

The results of molecular docking demonstrate the efficacy of curcumin and piperine against the target proteins. Additionally, analysis of the product resulting from the sonication procedure confirms the successful integration of curcumin and piperine into their nano formulations, maintaining their functional integrity. Future studies can address the comparison of the nano formulations using different methods, in terms of bioavailability, cytotoxicity and antibiofilm efficacy. The long-term effect of these phytochemicals on the biofilms in terms of substantivity of their antibacterial efficacy is worth studying.

CONCLUSION

Based on the results of this study, we can conclude that curcumin and piperine have been proven effective against the GTFs of *S. mutans* and *L. rhamnosus* through molecular docking. The nano formulation of curcumin and piperine using sonication method is an effective and simple method to obtain the benefits of the phytocompound without using any carriers.

REFERENCES:

1. Loesche, W.J. (1986). Role of Streptococcus mutans in human dental decay. Microbiological Reviews, 50(4), 353-380. https://doi.org/10.1128/mr.50.4.353-380.1986

2. Caufield, P.W., Schön, C.N., Saraithong, P., Li, Y., & Argimón, S. (2015). Oral Lactobacilli and Dental Caries: A Model for Niche Adaptation in Humans. Journal of Dental Research, 94(9 Suppl), 110S-8S. https://doi.org/10.1177/0022034515576052

3. Bowen, W.H., & Koo, H. (2011). Biology of Streptococcus mutans - Derived Glucosyltransferases: Role in Extracellular Matrix Formation of Cariogenic Biofilms. Caries Research, 45(1), 69–86. https://doi.org/10.1159/000324598

4. Schwendicke, F., Dörfer, C., Kneist, S., Meyer-Lueckel, H., & Paris, S. (2014). Cariogenic Effects of Probiotic Lactobacillus rhamnosus GG in a Dental Biofilm Model. Caries Research, 48(3), 186–192. https://doi.org/10.1159/000355907

5. Shrestha, L., Fan, H.M., Tao, H.R., & Huang, J.D. (2022). Recent Strategies to Combat Biofilms Using Antimicrobial Agents and Therapeutic Approaches. Pathogens, 11(3), 292. https://doi.org/10.3390/pathogens11030292

6. Avraham, M., Steinberg, D., Barak, T., Shalish, M., Feldman, M., & Sionov, R.V. (2023). Improved Anti-Biofilm Effect against the Oral Cariogenic Streptococcus mutans by Combined Triclosan/CBD Treatment. Biomedicines, 11(2), 521. https://doi.org/10.3390/biomedicines11020521

7. Ambareen, Z., & Chinappa, A. (2014). Go Green- Keep the Root Canal Clean!!! International Journal of Dental Sciences and Research, 2(6B), 21–25. https://doi.org/10.12691/ijdsr-2-6b-7

8. Sinha, D.J., & Sinha, A.A. (2014). Natural medicaments in dentistry. Ayu, 35(2), 113-118. https://doi.org/10.4103/0974-8520.146198

9. James, D. V., Balagopal, D., Charanya, D., .L, M., & S, N. (2023). Comparative Evaluation of Antimicrobial Efficacy of Aloe Barbadensis Miller, Curcuma Longa and Azadirachta Indica with Sodium Hypochlorite: An Invitro Study. *Journal of Biomedical and Pharmaceutical Research*, *11*(6). https://doi.org/10.32553/jbpr.v11i6.951

10. Dr. Gowtham S.P, Dr. Vandana J, Dr. Charanya C, Dr. Balagopal S, & Dr. Sunil CV. (2023). Evaluation of anti-bacterial efficacy of herbal irrigants against enterococcus faecalis using RT-PCR. Community practitioner, 20(09), 172–179. https://doi.org/10.5281/zenodo.8374788

11. Wong, J., Zou, T., Lee, A.H.C., & Zhang, C. (2021). The Potential Translational Applications of Nanoparticles in Endodontics. International Journal of Nanomedicine, 16, 2087-2106. https://doi.org/10.2147/IJN.S293518

12. Sohn, S.I., Priya, A., Balasubramaniam, B., Muthuramalingam, P., Sivasankar, C., Selvaraj, A., ... Pandian, S. (2021). Biomedical Applications and Bioavailability of Curcumin - An Updated Overview. Pharmaceutics, 13(12), 2102. https://doi.org/10.3390/pharmaceutics13122102

13. Tiwari, A., Mahadik, K.R., & Gabhe, S.Y. (2020). PIPERINE: A comprehensive review of methods of isolation, purification and biological properties. Medicine in Drug Discovery,7, 100027. https://doi.org/10.1016/j.medidd.2020.100027

14. Flora, G., Gupta, D., & Tiwari, A. (2013). Nanocurcumin: A promising therapeutic advancement over native curcumin. Critical Reviews in Therapeutic Drug Carrier Systems, 30(4), 331-368. https://doi.org/10.1615/critrevtherdrugcarriersyst.2013007236

15. Trigo Gutierrez, J. K., Zanatta, G. C., Ortega, A. L. M., Balastegui, M. I. C., Sanitá, P. V., Pavarina, A. C., et al. (2017). Encapsulation of curcumin in polymeric nanoparticles for antimicrobial Photodynamic Therapy. PLoS ONE, 12(11), e0187418. https://doi.org/10.1371/journal.pone.0187418

16. Ahrari, F., Mazhari, F., Ghazvini, K., Fekrazad, R., Menbari, S., & Nazifi, M. (2023). Antimicrobial photodynamic therapy against Lactobacillus casei using curcumin, nano-curcumin, or erythrosine and a dental LED curing device. Lasers in Medical Science, 38(1), 260. https://doi.org/10.1007/s10103-023-03914-y

17. Gopalakrishna, P.K., Jayaramu, R.A., Boregowda, S.S., Eshwar, S., Suresh, N.V., Abu Lila, A.S., Moin, A., Alotaibi, H.F., Obaidullah, A.J., & Khafagy, E.-S. (2023). Piperine-Loaded In Situ Gel: Formulation, In Vitro Characterization, and Clinical Evaluation against Periodontitis. Gels, 9(7), 577. https://doi.org/10.3390/gels9070577

18. Dhivya, S., & Rajalakshmi, A.N. (2018). A Review on the preparation methods of Curcumin Nanoparticles. PharmaTutor, 6(9), 6-10. https://doi.org/10.29161/PT.v6.i9.2018.6

19. Luo, H., Liang, D.F., Bao, M.Y., et al. (2017). In silico identification of potential inhibitors targeting Streptococcus mutans sortase A. International Journal of Oral Science, 9, 53–62. https://doi.org/10.1038/ijos.2016.58

20. Ren, Z., Chen, L., Li, J., & Li, Y. (2016). Inhibition of Streptococcus mutans polysaccharide synthesis by molecules targeting glycosyltransferase activity. Journal of Oral Microbiology, 8, 31095. https://doi.org/10.3402/jom.v8.31095

21. Nadkarni, M.A., Chen, Z., Wilkins, M.R., & Hunter, N. (2014). Comparative genome analysis of Lactobacillus rhamnosus clinical isolates from initial stages of dental pulp infection: identification of a new exopolysaccharide cluster. PLoS One, 9(3), e90643. https://doi.org/10.1371/journal.pone.0090643

22. Bertoncini-Silva, C., Vlad, A., Ricciarelli, R., Giacomo Fassini, P., Suen, V.M.M., & Zingg, J.-M. (2024). Enhancing the Bioavailability and Bioactivity of Curcumin for Disease Prevention and Treatment. Antioxidants, 13(3), 331. https://doi.org/10.3390/antiox13030331

23. Racz, L.Z., Racz, C.P., Pop, L.C., Tomoaia, G., Mocanu, A., Barbu, I., Sárközi, M., Roman, I., Avram, A., Tomoaia-Cotisel, M., & Toma, V.A. (2022). Strategies for Improving Bioavailability, Bioactivity, and Physical-Chemical Behavior of Curcumin. Molecules, 27(20), 6854. https://doi.org/10.3390/molecules27206854

24. Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., & Srinivas, P.S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Medica, 64(4), 353-356. https://doi.org/10.1055/s-2006-957450

25. Agu, P.C., Afiukwa, C.A., Orji, O.U., Ezeh, E.M., Ofoke, I.H., Ogbu, C.O., Ugwuja, E.I., & Aja, P.M. (2023). Molecular docking as a tool for the discovery of molecular targets of

nutraceuticals in diseases management. Scientific Reports, 13(1), 13398. https://doi.org/10.1038/s41598-023-40160-2

26. Uma Maheswari, K., & Sankar, S. (2024). In Silico Molecular Docking of Phytochemicals of Murraya koenigii Against Streptococcus mutans. Cureus, 16(2), e53679. https://doi.org/10.7759/cureus.53679

27. Mohanty, C., & Sahoo, S.K. (2010). The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Biomaterials, 31, 6597–6611. https://doi.org/10.1016/j.biomaterials.2010.04.062

28. Zheng, Z., Zhang, X., Carbo, D., Clark, C., Nathan, C., & Lvov, Y. (2010). Sonication-assisted synthesis of polyelectrolyte-coated curcumin nanoparticles. Langmuir, 26(11), 7679-7681. https://doi.org/10.1021/la101246a

29. Sandhuli, S.H., Dunuweera, S.P., Dunuweera, A.N., & Rajapakse, R.M.G. (2021). Synthesis of Curcumin Nanoparticles from Raw Turmeric Rhizome. ACS Omega. https://doi.org/10.1021/acsomega.0c06314

30. Ghosh, A., Banerjee, T., Bhandary, S., & Surolia, A. (2014). Formulation of nanotized curcumin and demonstration of its antimalarial efficacy. International Journal of Nanomedicine, 9, 5373-5387. https://doi.org/10.2147/IJN.S62756

31. Aziz, D. M., Hama, J. R., & Alam, S. M. (2015). Synthesising a novel derivative of piperine from black pepper (Piper nigrum L.). Journal of Food Measurement and Characterization, 9(3), 324–331. https://doi.org/10.1007/s11694-015-9239-2