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Dry and wet lab experiences of certain curcumin analogues' antibacterial effect on *S.aureus*

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ABSTRACT
Curcumin analogues are widely used in numerous biological disciplines. They are widely employed in the pharmaceutical sector and offer good pharmacology application prospects in the present era. In the current study, two curcumin analogues—1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (BDMPHDD)—were tested in vitro against the bacterial strain *S. aureus.* The testing demonstrated that the ligands may have antibacterial potential. The rule of five was used to pre-filter the compounds' drug-like characteristics prior to computer analysis. Then, to determine the mechanism by which the compounds limit the growth of S. aureus, molecular docking research was carried out using the AutoDock 4.2 tool. Six distinct target proteins from S. aureus were chosen for this purpose (PDB ID: 1T2P, 3U2D, 2W9S, 1N67, 2ZCO, and 4H8E). The target protein *Dihydrofolate reductase* enzyme (PDB ID: 2W9S) and *Staphylococcus aureus sortase-A* (PDB ID: 1T2P) demonstrated a good binding affinity for the two analogues, (BDMPHDD) & (DPHDD) respectively.Due to the inactivation of these enzymes, the substances 1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (BDMPHDD) exhibit significant growth-inhibitory potential against *S. aureus*.

Key Words: Curcumin analogue, Antibacterial study, Molecular docking

1. Introduction
Therapeutic research is crucial for lowering the incidence of human diseases and improving human quality of life. Pathogenic organisms are responsible for many diseases. One of these multi-drug-resistant bacteria is *S. aureus*. This bacterium is naturally present on the skin and in the nasopharynx of humans. Infections of the skin, vagina, nose, urethra and digestive system can be brought on by *S. aureus*[1,2]. Although there are many different antibiotics and chemotherapeutic drugs available to treat these bacteria, due to their high cost, only individuals with severely resistant strains should use them. The development of innovative and potent chemotherapy medications is therefore essential for the medical sector.

2. Materials and methods
2.1 In silico molecular docking studies
The curcumin analogues in MOL format were determined to have certain structures using the Chem Sketch program and open babel software was used to transfer the structure to PDB format. Protein structures were retrieved from RCSB PDB in PDB format. Hydrogen atoms were added and the proteins' existing ligands and water molecules were removed using Pymol software before being saved in PDB format.

2.1.1 Lipinski rule of five: The Lipinski rule states that an orally active drug will be small and slightly

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lipophilic[3]. A drug has strong oral activity if it satisfies the five criteria in this criterion, which emphasizes molecular traits over pharmacological action.

2.1.2 Molecular docking: To determine the mechanism through which the curcumin analogues inhibit bacterial growth, docking tests were conducted. The interactions and binding affinities of these drugs with different target proteins in *S. aureus* were analysed using docking experiments[4]. The PDB IDs for the chosen target molecules were 1T2P, 3U2D, 2W9S, 1N67, 2ZCO, and 4H8E[5]. The protein—curcumin analogue adducts' binding energies were learned using molecular docking calculations using the Auto Dock 4.2 programme [6-9]. The software BIOVIA Discovery Studio was used to build the protein-ligand complexes' 3D and 2D interaction graphs.

2.2 Preparation and Characterization of Curcumin analogues

Aldehydes (benzaldehyde and 3,4-dimethoxy benzaldehyde) were combined with an acetylacetone-boric oxide complex to form curcuminoid analogues in an ethyl acetate medium with tributyl borate and n-butyl amine. In order to get pure crystalline content, the products were refined using a 5:1 (v/v) chloroform:acetone combination as the eluent in column chromatography over silica gel (60-120 mesh)[10].IR, ^{13}C NMR, ^{1}H NMR, and mass spectral methods are used to characterize the ligands.[11]

2.3 In vitro Antibacterial Studies

The antibacterial activity of the test material is usually evaluated using the Agar well diffusion method[12]. On identically sized glass petri plates, Mueller-Hinton agar[13] (15–20 mL) was added and left to set. A uniform inoculum of the test organism was applied to the surface of the plates using a sterile cotton swab. Four 8 mm-diameter wells that were 20 mm apart were aseptically punched into each plate using a sterile cork borer. The test sample (40 and 80 L) from the 10 mg/ml stock was filled into wells T1 and T2. As a positive and negative control, gentamycin (40 l from a 4 mg/ml stock) and the solvent used for sample dilution, respectively, were added. The plates were incubated for 24 hours in an aerobic environment at 36°C + 1°C. After incubation, the plates were examined, and the mm-sized zone that inhibited bacterial growth around the wells was measured[14].

3. Result and Discussion

The compounds were initially pre-filtered using Lipinski's rule of five to check for drug-like characteristics. As determined by the rule, the two analogues' characteristics, including their masses, hydrogen bond donors and acceptors, log P (the octanol-water partition coefficient), and molar refractivity, are displayed in Table 1. A drug that is active when taken orally must have fewer than two infractions [15]. Findings indicated that neither molecule violates the Lipinski rule, indicating that they have the potential to behave as active drugs that can be taken orally.

It is now crucial to comprehend the process by which the chemicals prevent bacterial development. Molecular docking studies were performed to identify which protein target in bacteria the ligands have the highest binding affinity for. The stability of the protein-ligand complex was assessed using the highest binding energy, lowest inhibition constant and the number of interactions between the ligand and the active site residues. Protein and ligands frequently interact via electrostatic interactions such as pi-anion interactions, van der Waals interactions, and unfavourable pi-donor interactions. Moreover, Hydrogen bond interactions include conventional and non-conventional H bonds, and hydrophobic interactions include pi-sigma, alkyl and pi-alkyl interactions are also seen between protein and ligand. The binding affinity of the compound with the target protein is the result of all the interactions and binding energy existing between them. The docking scores and the number of interactions of the ligands with protein models under study were enlisted in Table 2.

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Table 1 Lipinski Rule of Five

Lipinski rule of five			
Parameters	**Ligands**	**Conditions for Druglike property**	
	dphdd	**bdmphdd**	
Molecular weight g/mol	276.33	396.43	< 500
H- Bond Donor	1	1	< 5
H-Bond Acceptor	2	6	< 10
log P value	3.96	3.91	< 5
Molar Refractivity	86.67	112.64	40–130

Table 2 Docking scores and No.of Interactions

Active Target	2W9S		1N67		1T2P		2ZCO		3U2D		4H8E	
Ligands	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd	dphdd	bdmphdd
Active Site and Run	9	1	2	1	7	3	3	1	2	9	3	6
BE(kcal/mol)	-8.7	-9.4	-8.5	-4.96	-9.8	-7.48	-7.62	-6.73	-7.89	-7.3	-9.4	-8.4
Ligand efficiency	-0.4	-0.3	-0.4	-0.17	-0.5	-0.26	-0.36	-0.23	-0.38	-0.3	-0.45	-0.3
inhib constant (nM)	394	120	758.6	231.2	69.5	3.27	2.6	11.6	1.65	4.66	128.6	692
intermolar energy	-11	-12	-10.1	-7.94	-12	-10.5	-9.41	-9.71	-9.68	-10	-11.2	-11
vdw hb disolv energy	-10	-12	-10.1	-7.97	-12	-9.92	-9.39	-9.78	-9.73	-10	-11.1	-11
electrostatic energy	-0.1	-0.1	-0.07	0.02	-0	-0.54	-0.02	0.07	0.05	-0.1	-0.07	-0
total internal	-0.9	-1.2	-1.18	-1.78	-1.2	-1.85	-1.31	-1.94	-1.41	-1.9	-0.7	-1.7
torsional energy	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98	1.79	2.98
unbound energy	-0.9	-1.2	-1.18	-1.78	-1.2	-1.85	-1.31	-1.94	-1.41	-1.9	-0.7	-1.7
cRMS	0	0	0	0	0	0	0	0	0	0	0	0
refRMS	40.8	39	81.12	86.97	24.4	39.1	78.49	57.6	25	24	26.29	23.7
rseed1	None	None	None	None	None	None	None	None	None	None	None	None
rseed2	None	None	None	None	None	None	None	None	None	None	None	None
Van der Waals	13	14	12	13	11	4	11	8	6	7	11	12
Hydrogen bond	1	5	2	2	2	2	0	1	2	2	2	3
Others	5	3	3	5	6	13	7	6	6	10	5	14
Total Interactions	19	22	17	20	19	19	18	15	14	19	18	29

The following Figures showing the Binding pockets and Protein Ligand Interactions.

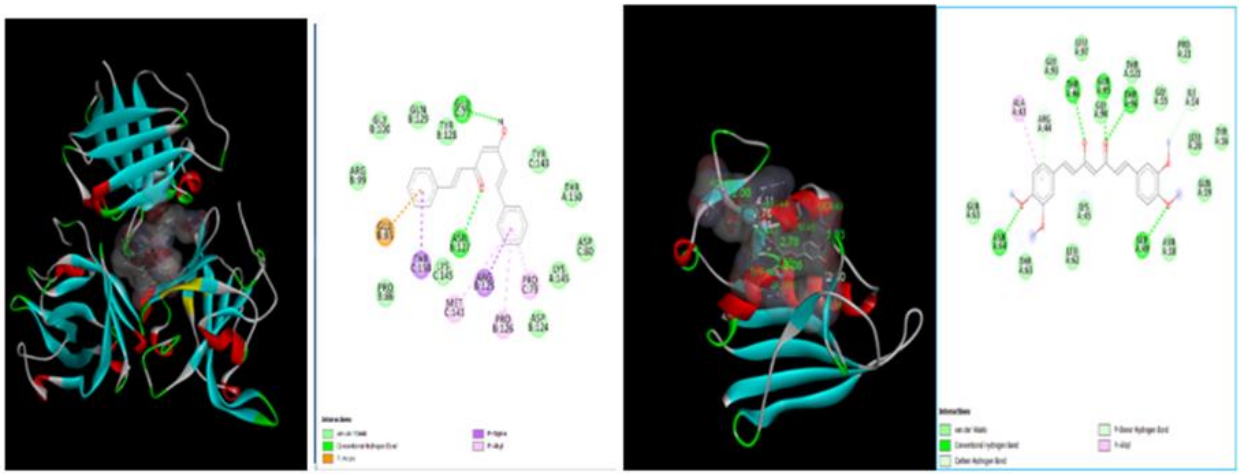

Fig 1 Binding pockets and protein – Ligand Interaction

The curcumin analogues were synthesized in the wet lab by Pabon's Method and the characterization by various spectral analyses was conducted. Spectral Data are shown in Tables 3,4,5 and 6.

Table 3 IR Data

DPHDD	BDMPHDD	Probable IR Assaignments
3040	2929	ν Enolic
1622	1620	ν (C=O)Chelated
1581	1585	ν (C=C)Phenyl
1512	1507	ν (C-C)Alkenyl
1456	1466	ν_{as}(C-C-C)Chelate ring
1426	1423	ν_{s}(C-C-C)Chelate ring
1145	1121	ν β(C-H)Chelate ring
968	958	ν (CH=CH)trans

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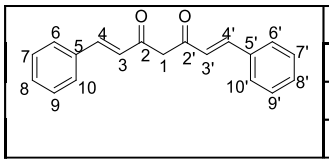
Table 4 ^{1}H NMR and Mass Spectral Data

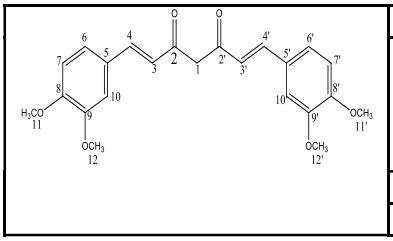
Ligands	Chemical shift(δ) in ppm					Mass spectral data (m/z)
	Enolic	Methine	Alkenyl	Phenyl	Substituent	
DPHDD	10.014	6.78	6.98-7.79	7.31-7.60	---	276,199,173,145,131,103,90,77
BDMPHDD	9.84	6.75	6.81-7.61	7.06-7.14	3.85 (methoxy)	396,259,233,205,191,137,163

Table 5 ^{13}C NMR Data of DPHDD



C1	C2,C2'	C3,C	C4,C4'	C5,C5'
98.63	200.99	130.26	130.26	133.76
C6,C6'	**C7,C7'**	**C8,C8'**	**C9,C9'**	**C10,C10'**
128.57	128.57	128.57	128.57	128.57

Table 6 ^{13}C NMR Data of BDMPHD



C1	C2,C2'	C3,C3'	C4,C4'	C5,C5'	C6,C6'
101.38	191.06	128.75	140.51	122.74	122.11
C7,C7'	**C8,C8'**	**C9,C9'**	**C10,C10'**	**C11,C11'**	**C12,C12'**
111.21	149.31	151.13	109.81	56.07	55.99

The ligands BDMPHDD & DPHDD have the potential to function as effective antibacterial agents, according to an in vitro antibacterial investigation (Fig 2). Despite having less action than the common antibiotic gentamycin, both BDMPHDD and DPHDD have noticeable growth-inhibitory potential. At an 80 l well-1 concentration, gentamycin's zone of inhibition on S. aureus measured 22 mm in diameter, whereas the analogues BDMPHDD & DPHDD measured 11 mm & 13 mm, respectively. It was discovered that the zone of inhibition grew larger as the chemical concentration did. They can therefore be regarded as effective antibacterial agents against *S. aureus*.

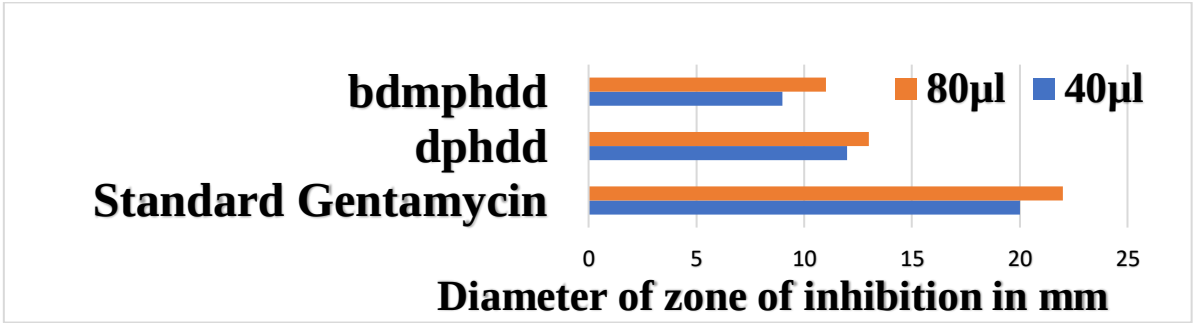


Fig 2 Antibacterial Effects of Curcumin Analogues

Conclusion

The two curcumin analogues 1,7-diphenylhepta-1,6-diene-3,5-dione (DPHDD) and 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (BDMPHDD) compare favourably to the conventional antibiotic gentamycin in terms of their ability to prevent the growth of the harmful bacterium *S.aureus*. At a concentration of 80 µl well^{-1} , DPHDD and BDMPHDD, respectively, demonstrated maximum zones of inhibition of about 13 mm and 11 mm. Both follow Lipinski's rule of five and have features like those of drugs. Target proteins *Dihydrofolate*

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reductase enzyme (PDB ID: 2W9S) and *Staphylococcus aureus sortase-A* (PDB ID: 1T2P) showed excellent binding affinity for the two analogues, (BDMPHDD) & (DPHDD), respectively. They unambiguously show that the inactivation of the enzymes *Dihydrofolate reductase* and *Staphylococcus aureus sortase-A* is the primary cause of the significant growth-inhibitory capacity of these curcumin analogues against the pathogenic bacterium S. aureus.. These results are found to be useful for further in vivo analysis.

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