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Computational multi-target approach to target essential enzymes of *Leishmania donovani* using comparative molecular dynamic simulations and MMPBSA analysis

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Abstract

Introduction: Visceral leishmaniasis (VL) is caused by *Leishmania donovani*. The purine and pyrimidine pathways are essential for *L. donovani*. Simultaneously inhibiting multiple targets could be an effective strategy to eliminate the pathogen and treat VL.

Objective: We aimed to target the essential enzymes of *L. donovani* and inhibit them using a multi-target approach.

Materials and methods: A systematic analytical method was followed, in which first reported inhibitors of two essential enzymes (adenine phosphoribosyl-transferase [APRT] and dihydroorotate dehydrogenase [DHODH]) were collected and then ADMET and PASS analyses were conducted using the Lipinski rule and Veber's rule. Additionally, molecular docking between screened ligands and proteins were performed. The stability of complexes was analyzed using molecular dynamics (MD) simulations and MMPBSA analysis.

Results: Initially, 6,220 unique molecules were collected from the PubChem database, and then the Lipinski rule and Veber's rule were used for screening. In total, 203 compounds passed the ADMET test; their antileishmanial properties were tested by PASS analysis. As a result, 15 ligands were identified. Molecular docking simulations between APRT or DHODH and these 15 ligands were performed. Four molecules were found to be plant-derived compounds. Lig_2 and Lig_3 had good docking scores with both proteins. MD simulations were performed to determine the dynamic behavior and binding patterns of complexes. Both MD simulations and MMPBSA analysis showed Lig_3 is a promising antileishmanial inhibitor of both targets.

Conclusion: Promising plant-derived compounds that might be used to combat VL were obtained through a multi-target approach.

KEYWORDS

adenine phosphoribosyl-transferase, dihydroorotate dehydrogenase, *Leishmania donovani*, MD simulation, multi-target, visceral leishmaniasis

is a derivative of Mammea A/AA, which is a natural product present in *Mesua racemose*; it was reported to act as an antiparasitic polyphenolic drug.[63] At last, Lig_4 is a flavonoid which is known as Rubraflavone A.[37] Selected compounds bound to the active sites and showed interactions with specific residues, changing the activity of APRT and DHODH. Ligands with lower binding affinity in both proteins displayed hydrogen bonding and non-bonded interactions with amino acid residues.

Docking provides only the binding energy of the protein–ligand complexes; to know whether the interactions are stable for a longer period, MD simulations were performed. Biomolecular conformational dynamics of the protein–ligand complexes were analyzed by computational MD simulations. Data generated from simulations were later used to examine the intermolecular forces in protein–ligand complexes.

The novelty of our work lies in the utilization of a multi-target approach. We investigated the effects of predicted antileishmanial molecules on both APRT and DHODH. By using different filters such as Lipinski's rule of five, Veber's rule, ADMET and PASS analysis, set of 6220 compounds were processed. After the screening process, a total of 15 compounds were selected, which were later used to construct complexes with APRT and DHODH, the drug target proteins of L. donovani, i.e., a total of 30 protein–ligand complexes. Furthermore, we analyzed the stability and confirmation of ligand–protein complexes. First, a short simulation was performed to reduce the number of ligands. MD simulations of APRT and DHODH with four compounds for both proteins and one specific inhibitor for each protein showed that Lig_2 and Lig_3 formed considerably stable complexes with both proteins. RMSD and Rg analyses of the complexes provided information regarding proper binding of molecules to the proteins. In the second phase, validation based on long MD simulations of 100 ns was performed for the six protein–drug complexes to ensure that short simulations did not provide biased results. It was observed that the apo protein of APRT showed fluctuations in RMSD and Rg compared to complex structures, whereas the apo protein of DHODH showed minor fluctuations. Lig_3 showed a higher number of H-bonds with APRT and DHODH than Lig_2 and inhibitor until the end of the simulation, suggesting that these interactions are more stable. Analysis of non-bonded contacts between the two proteins and ligands revealed that there are contacts other than H-bonds, like van der Waals bonds and hydrophobic interactions, which contribute to the interactions. Therefore, we infer that Lig_3 preferably stays inside the binding pocket of the protein. No significant confirmational change was observed for APRT and DHODH upon binding with molecules.

In addition to the MD simulation-based investigation, MMPBSA analysis was performed to provide additional support to earlier observations. The total binding energies of the compounds with the proteins were obtained. We observed that APRT–Lig_3 and DHODH–Lig_3 showed higher binding energy than other complexes. The larger contribution of electrostatic energy means that H-bonds are higher in number, thus favoring and influencing the binding complexes, which is in agreement with the results of the H-bond analysis of MD simulations. Due to the high number of H-bonds, the binding energy between the protein and ligand increases, thus supporting the formation of a stable configuration.

Interestingly, per-residue free energy decomposition analysis highlighted ligand binding spots, and it revealed that the contributions of specific residues were quite high, which ultimately provides significant input to the binding energy, especially in the case of APRT–Lig_3 and DHODH–Lig_3. Furthermore, the high electrostatic energy present in both complexes with Lig_3 indicated that the H-bonds were present until the end of the simulation and thus played a notable role. van der Waals bonds and hydrophobic interactions also contributed to stabilizing the complexes.

This theoretical study highlights that stable interactions between a compound and different proteins may have the ability to inhibit both proteins. Moreover, details of proteins, protein–ligand complexes, residues forming the active sites, and different poses of proteins were obtained, which underlined the behavior of proteins and ligands in the artificial cell-like environment. Our proposed hit may assist in the development of new antileishmanial drugs targeting essential proteins, preventing transmission, and supporting eradication of the disease.

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DATA AVAILABILITY STATEMENT

The data used to support the results of this study are given in the manuscript and supplementary section.

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REFERENCES

1. World Health Organization: Weekly Epidemiological Record (WER); 2016.
2. Ruiz-Postigo JA, Jain S, Mikhailov A, Valadas S, Warusavitha S, Osman M. Global leishmaniasis surveillance: 2019–2020, a baseline for the 2030 roadmap. *Wkly Epidemiol Rec*. 2021;96:401-419.
3. Akhoundi M, Kuhls K, Cannet A, et al. A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. *PLoS Negl Trop Dis*. 2016;10(3):e0004349. doi:10.1371/journal.pntd.0004349
4. WHO. Epidemiological situation. WHO. Accessed December 20, 2021. https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis
5. Pulvertaft RJ, Hoyle GF. Stages in the life-cycle of Leishmania donovani. *Trans R Soc Trop Med Hyg*. 1960;54(2):191-196. doi:10.1016/0035-9203(60)90057-2
6. Boelaert M, Criel B, Leeuwenburg J, Van Damme W, Le Ray D, Van der Stuyft P. Visceral leishmaniasis control: a public health perspective. *Trans R Soc Trop Med Hyg*. 2000;94(5):465-471. doi:10.1016/s0035-9203(00)90055-5
7. Rajkhowa S, Hazarika Z, Jha AN. Systems biology and bioinformatics approaches in leishmaniasis. *Appl Nanobiotech Neglect Trop Dis Elsevier*. 2021;509-548. doi:10.1016/B978-0-12-821100-7.00018-2

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Molecular scaffold recognition of drug molecules against essential genes of *Leishmania donovani* using biocomputing approach

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ABSTRACT

Leishmania donovani, an obligatory intracellular flagellate pathogen, is the underlying cause of visceral leishmaniasis (VL), a fatal disease that poses a significant challenge to existing therapeutic approaches and leads to human mortality. In an endeavor to find an antileishmanial drug to combat VL, we aimed to assess the approved drug molecules against the specific drug targets of VL. In this study, a theoretical method was used to select two essential therapeutic targets (pyridoxal kinase [PK] and sterol alpha-14 demethylase [SDM]) which were present in both the data set of essential genes and drug target proteins. The selected PK and SDM proteins in *L. donovani* play pivotal roles as essential enzymes in the crucial vitamin B6 salvage and sterol biosynthesis pathways, respectively, leading to pathogenicity in humans. In addition to that drugs were gathered from the DrugBank and Drug Central databases and 325 (out of 4867) compounds having anti-parasitic properties were screened by PASS analysis. Consequently, three ligands (referred to as Lig_1, Lig_2, and Lig_3) were chosen based on their elevated Pa values, docking scores, and notable medicinal applications. Moreover, the result obtained from MD simulation suggests Lig_1 [Nitazoxanide (PubChem ID-41684)] does not affect the structural integrity of both targets. Additionally, evaluation of total binding energies by MMPBSA analysis showed stronger binding of Lig_1 with PK and SDM is -100.71 and -175.61 kJ/mol, respectively compared to others. As a whole, the methodology employed in this research involves the simultaneous identification of suitable protein targets and potential inhibitors. Through this investigation, we have demonstrated that compounds derived from a biocomputing approach exhibit interaction mechanisms as inhibitors against drug targets, offering a promising avenue for addressing VL.

1. Introduction

Leishmaniasis, a disease transmitted by vectors, is caused by protozoa characterized by a hemoflagellate structure. This ailment poses a significant global public health challenge as a zoonotic condition. It is categorized as a neglected tropical disease due to the insufficient focus on its infection. The impact of the *Leishmania* pathogen is predominantly evident in countries such as India, Nepal, Bangladesh, Ethiopia, Sudan, Kenya, and others. These nations, mostly characterized as underdeveloped, experience substantial repercussions, thereby influencing their progress and development. Furthermore, these parasites are prevalent in countries situated within tropical and temperate zones. (Croft and Coombs, 2003). The World Health Organization (WHO) has documented *Leishmania* parasite infections in approximately 12 million individuals. The most severe and lethal form of leishmaniasis is visceral leishmaniasis (VL), better known as kala-azar in India (Fernandes et al., 2013). The primary cause of visceral leishmaniasis (VL) is attributed to *Leishmania donovani*, a protozoan organism that exists in two distinct forms: promastigote and amastigote. *L. donovani* needs both humans and sand flies as hosts for the successful completion of life cycle. In this context, when an infected sandfly bites a human, it transmits the pathogen into the human body, enabling the completion of the remaining portion of its life cycle. During this life cycle phase, the parasites start exerting their impact on humans, ultimately resulting in the demise of the host (Ready, 2013; Singh et al., 2015). This pathogen's life cycle gives information about the infective stage, which aids in prevention and development of therapies against the pathogen.

The treatment of visceral leishmaniasis relies solely on chemical compounds as medications. The present therapeutic options for leishmaniasis encompass miltefosine, paromomycin, and amphotericin B (eBioMedicine, 2023). To develop new medications, diverse methodologies have been introduced over time, including systems biology approaches (Rajkhowa et al., 2021), kinetic modeling approaches (Bora and Jha, 2020), multi-target approaches (Saha and Nath Jha, 2023) etc. Various fields within computational biology have emerged, providing numerous avenues for exploration, in the quest to discover efficient medications that combat diseases such as cancer, malaria, COVID-19, and more (Bora and Nath Jha, 2019; Indari et al., 2022).

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Table 4
Average MMPBSA energy (kJ/mol) for the binding of PK and SDM with selected ligands.

System	PK-Lig_1	PK-Lig_2	PK-Lig_3	SDM-Lig_1	SDM-Lig_2	SDM-Lig_3
$G_{Binding}$ (**w + x + y + z**)	−100.71 ± 22.01	−73.23 ± 30.80	−101.79 ± 18.97	−175.61 ± 12.64	−131.93 ± 12.73	−125.25 ± 12.91
G_{vdW} (**w**)	−140.05 ± 11.42	−139.33 ± 12.04	−151.65 ± 11.1	−180.69 ± 10.31	−148.98 ± 9.81	−154.39 ± 8.41
G_{elec} (**x**)	−142.44 ± 24.45	−78.86 ± 27.86	−77.00 ± 22.28	−127.99 ± 16.79	−39.95 ± 15.64	−46.47 ± 9.44
G_{pol} (**y**)	197.61 ± 15.19	162.21 ± 22.12	142.00 ± 19.36	149.76 ± 9.53	75.32 ± 8.30	91.74 ± 9.20
G_{SASA} (**z**)	−15.83 ± 0.71	−17.25 ± 0.99	−15.15 ± 0.73	−16.68 ± 0.72	−18.32 ± 0.89	−16.13 ± 0.68

resistance developed against existing medications. Within this investigation, a computational framework was employed to identify two druggable targets that exhibited stronger binding affinity to three compounds. Subsequent molecular dynamics (MD) simulations and MMPBSA analysis uncovered that Lig_1 (Nitazoxanide) not only preserves the structural integrity of both proteins but also enhances the stability essential for inhibiting PK and SDM targets. In general, the study underscores the importance of stability, interactions, and binding energies between the compounds and selected crucial proteins in altering the functions of both targets and ultimately leading to their inhibition. In a nutshell, the findings indicate that obtain compound may exhibit structural mechanism of inhibition against critical PK and SDM proteins of *L. donovani.* Additional improvements and advancements are required to evaluate the molecule's efficacy *in vitro* and *in vivo* against these particular targets.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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References

Abraham, M.J., Murtola, T., Schulz, R., Páll, S., Smith, J.C., Hess, B., Lindahl, E., 2015. GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1, 19–25.
Are, S., Gatreddi, S., Jakkula, P., Qureshi, I.A., 2020. Structural attributes and substrate specificity of pyridoxal kinase from *Leishmania* donovani. Int. J. Biol. Macromol. 152, 812–827.
Avram, S., Bologa, C.G., Holmes, J., Bocci, G., Wilson, T.B., Nguyen, D.T., Curpan, R., Halip, L., Bora, A., Yang, J.J., Knockel, J., Sirimulla, S., Ursu, O., Oprea, T.I., 2021. DrugCentral 2021 supports drug discovery and repositioning. Nucleic. Acids. Res. 49 (D1), D1160–D1169.
Bora, N., Jha, A.N., 2020. *In silico* metabolic pathway analysis identifying target against Leishmaniasis - a kinetic modeling approach. Front. Genet. 11, 179.
Bora, N., Nath Jha, A., 2019. An integrative approach using systems biology, mutational analysis with molecular dynamics simulation to challenge the functionality of a target protein. Chem. Biol. Drug Des. 93 (6), 1050–1060.
Bussi, G., Donadio, D., Parrinello, M., 2007. Canonical sampling through velocity rescaling. J. Chem. Phys. 126 (1), 014101.
Croft, S.L., Coombs, G.H., 2003. Leishmaniasis–current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol. 19 (11), 502–508.
eBioMedicine, 2023. Leishmania: an urgent need for new treatments. EBioMedicine 87, 104440.
Eliot, A.C., Kirsch, J.F., 2004. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu. Rev. Biochem. 73 (1), 383–415.
Fernandes, F.R., Ferreira, W.A., Campos, M.A., Ramos, G.S., Kato, K.C., Almeida, G.G., Correa, J.D.J., Melo, M.N., Demicheli, C., Frezard, F., 2013. Amphiphilic antimony(V) complexes for oral treatment of visceral Leishmaniasis. Antimicrob. Agents Chemother. 57 (9), 4229–4236.
Ghaffarifar, F., Esavand Heydari, F., Dalimi, A., Hassan, Z.M., Delavari, M., Mikaeiloo, H., 2015. Evaluation of apoptotic and antileishmanial activities of artemisinin on promastigotes and BALB/C mice infected with leishmania major. Iran. J. Parasitol. 10, 258–267.
Ghosh, M., Roy, K., Das Mukherjee, D., Chakrabarti, G., Roy Choudhury, K., Roy, S., 2014. Leishmania donovani infection enhances lateral mobility of macrophage membrane protein which is reversed by liposomal cholesterol. PLoS Negl. Trop. Dis. 8 (12), e3367.
Hess, B., 2008. P-LINCS: a parallel linear constraint solver for molecular simulation. J. Chem. Theory Comput. 4 (1), 116–122.
Ikram, N., Simonsen, H.T., 2017. A review of biotechnological artemisinin production in plants. Front. Plant Sci. 8, 1966.
Indari, O., Kumar Singh, A., Tiwari, D., Chandra Jha, H., Nath Jha, A., 2022. Deciphering antiviral efficacy of malaria box compounds against malaria exacerbating viral pathogens- Epstein Barr virus and SARS-CoV-2, an in silico study. Med. Drug Discov. 16, 100146.
Brindha, J., Balamurali, M.M., Chanda, K., 2021. An overview on the therapeutics of neglected infectious diseases-Leishmaniasis and chagas diseases. Front. Chem. 9, 622286.
Jakhmola, S., Hazarika, Z., Jha, A.N., Jha, H.C., 2022. In silico analysis of antiviral phytochemicals efficacy against Epstein-Barr virus glycoprotein H. J. Biomol. Struct. Dyn. 40 (12), 5372–5385.
Jha, A.N., 2023. Computational approaches to build therapeutic paradigms targeting genes, proteins and pathways against neglected tropical diseases (NTDs). Front. Genet. 14, 1183034.
Jones, D.C., Alphey, M.S., Wyllie, S., Fairlamb, A.H., 2012. Chemical, genetic and structural assessment of pyridoxal kinase as a drug target in the African trypanosome. Mol. Microbiol. 86 (1), 51–64.
Katiki, M., Sharma, M., Neetu, N., Rentala, M., Kumar, P., 2023. Biophysical and modeling-based approach for the identification of inhibitors against DOHH from Leishmania donovani. Brief Funct. Genom. 22, 217–226.
Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J., Bryant, S.H., 2016. PubChem substance and compound databases. Nucleic. Acids. Res. 44 (D1), D1202–D1213.
Kronenberger, T., Lindner, J., Meissner, K.A., Zimbres, F.M., Coronado, M.A., Sauer, F.M., Schettert, I., Wrenger, C., 2014. Vitamin B6-dependent enzymes in the human malaria parasite Plasmodium falciparum: a druggable target? Biomed. Res. Int. 2014, 108516.
Kumar, V., Sharma, M., Rakesh, B.R., Malik, C.K., Neelagiri, S., Neerupudi, K.B., Garg, P., Singh, S., 2018. Pyridoxal kinase: a vitamin B6 salvage pathway enzyme from Leishmania donovani. Int. J. Biol. Macromol. 119, 320–334.
Kumari, I., Lakhanpal, D., Swargam, S., Nath Jha, A., 2023. Leishmaniasis: omics approaches to understand its biology from molecule to cell level. Curr. Protein Pept. Sci. 24 (3), 229–239.
Kumari, R., Kumar, R., Open Source Drug Discovery Consortium, Lynn, A., 2014. g_mmpbsa–a GROMACS tool for high-throughput MM-PBSA calculations. J. Chem. Inf. Model. 54 (7), 1951–1962.
Lagunin, A., Stepanchikova, A., Filimonov, D., Poroikov, V., 2000. PASS: prediction of activity spectra for biologically active substances. Bioinformatics 16 (8), 747–748.
Lazarou, R., Heinrich, M., 2019. Herbal medicine: who cares? The changing views on medicinal plants and their roles in British lifestyle. Phytother. Res. 33, 2409–2420.
Lepesheva, G.I., Waterman, M.R., 2011. Sterol 14alpha-demethylase (CYP51) as a therapeutic target for human trypanosomiasis and Leishmaniasis. Curr. Top. Med. Chem. 11, 2060–2071.
Lin, Z., van Gunsteren, W.F., 2013. Refinement of the application of the GROMOS 54A7 force field to beta-peptides. J. Comput. Chem. 34 (32), 2796–2805.
Lyndem, S., Hazarika, U., Athul, P., Bhatta, A., Prakash, V., Jha, A.N., Roy, A.S., 2023. A comprehensive *in vitro* exploration into the interaction mechanism of coumarin derivatives with bovine hemoglobin: spectroscopic and computational methods. J. Photochem. Photobiol. A Chem. 436, 114425.
McCall, L.I., El Aroussi, A., Choi, J.Y., Vieira, D.F., De Muylder, G., Johnston, J.B., Chen, S., Kellar, D., Siqueira-Neto, J.L., Roush, W.R., Podust, L.M., McKerrow, J.H., 2015. Targeting ergosterol biosynthesis in Leishmania donovani: essentiality of sterol 14 alpha-demethylase. PLoS Negl. Trop. Dis. 9 (3), e0003588.
O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open babel: an open chemical toolbox. J. Cheminform. 3 (1), 33.
Oliveira-da-Silva, J.A., Machado, A.S., Ramos, F.F., Tavares, G.S.V., Lage, D.P., Ludolf, F., Steiner, B.T., Reis, T.A.R., Santos, T.T.O., Costa, L.E., Martins, V.T., Galvani, N.C.,

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ORIGINAL PAPER

Integrated subtractive genomics and structure-based approach to unravel the therapeutic drug target of *Leishmania* species

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Abstract

Leishmaniasis is a complex vector-borne disease caused by intracellular protozoan parasites of the *Leishmania* genus. It presents a significant public health challenge in tropical and subtropical regions globally. As resistance to treatment increases, managing and controlling Leishmaniasis becomes more challenging, necessitating innovative approaches. To address this challenge, our study utilized subtractive genomics and structure-based approaches to identify common drug targets and combat antimicrobial resistance (AMR) across five *Leishmania* species strains. The subtractive genomics approach unraveled Glutamate Dehydrogenase (GDH) as a promising drug target for treating Leishmania infections. The investigation considered established methodologies observed in analogous studies, orthologous group, and druggability tests. Multiple sequence alignment revealed conserved sequences in GDH, while phylogenetic tree analysis provided insights into the evolutionary origin and close relationships of GDH across *Leishmania* species. Conserved sequences in GDH along with its function in pathogenicity provided insights into the close relationships of GDH across *Leishmania* species. Using a structure-based approach, our study showed the molecular interactions between GDH and three ligands—Bithionol, GW5074, and Hexachlorophene—through molecular docking and 100 ns molecular dynamics (MD) simulations. GW5074 exhibited a significant affinity for GDH, as indicated by stable RMSD values, a more compact conformation, and a higher number of hydrogen bonds than Bithionol. MMPBSA analysis confirmed the superior binding energy of the GW5074-GDH complex, emphasizing its potential as a potent ligand for drug development. This comprehensive analysis identified GW5074 as a promising candidate for inhibiting GDH activities in *Leishmania* species, contributing to the development of effective therapeutics against *Leishmania* infections.

Keywords Leishmaniasis · Subtractive genomics · Glutamate dehydrogenase · Structure-based approach · MD simulations

Abbreviations

AMR	Antimicrobial resistance
GDH	Glutamate Dehydrogenase
MD	Molecular Dynamics
CL	Cutaneous leishmaniasis
MCL	Mucocutaneous leishmaniasis
VL	Visceral leishmaniasis
WHO	World Health Organization
NCBI	National Centre for Biotechnology Information
MM/PBSA	Molecular Mechanics Poisson–Boltzmann Surface Area
TCA	Tricarboxylic acid
MSA	Multiple Sequence Alignment
MST	3-Mercaptopyruvate sulfurtransferase
IDH	Isocitrate dehydrogenase
UGE	UDP-glucose 4 epimerase
GDH	Glutamate dehydrogenase
DDH	Dihydroorotate dehydrogenase
RMSD	Root Mean Square Deviations
Rg	Radius of gyration
RMSF	Root Mean Square Fluctuations

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Khan K, Jalal K, Khan A, Al-Harrasi A, Uddin R (2021) Comparative metabolic pathways analysis and subtractive genomics profiling to prioritize potential drug targets against Streptococcus pneumoniae. Front Microbiol 12:796363

Kumari R, Kumar R, Open Source Drug Discovery Consortium, Lynn A (2014) g_mmpbsa—A GROMACS tool for high-throughput MM-PBSA calculations. J Chem Inf Model 54(7):1951–1962

Lyndem S, Hazarika U, Bhatta A, Prakash V, Jha AN, Roy AS (2023) In vitro interactions of esculin and esculetin with bovine hemoglobin alter its structure and inhibit aggregation: insights from spectroscopic and computational studies. New J Chem 47(30):14447–14468

Marcili A, Speranca MA, da Costa AP, Madeira Mde F, Soares HS, Sanches Cde O et al (2014) Phylogenetic relationships of Leishmania species based on trypanosomatid barcode (SSU rDNA) and gGAPDH genes: Taxonomic revision of Leishmania (L.) infantum chagasi in South America. Infect Genet Evol 25:44–51

Morris MG, Goodsell SD, Pique EM, et al. (2014) AutoDock version 4.2; updated for version 4.2.6. SL: The Scripps Research Institute.

Pathan EK, Kulkarni AM, Prasanna NVL, Ramana CV (1864) Deshpande MV (2020) NADP-dependent glutamate dehydrogenases in a dimorphic zygomycete Benjaminiella poitrasii: Purification, characterization and their evaluation as an antifungal drug target. Biochim Biophys Acta Gen Subj 11:129696

Prado L, Giacchetto Felice A, Rodrigues TCV, Tiwari S, Andrade BS, Kato RB et al (2022) New putative therapeutic targets against Serratia marcescens using reverse vaccinology and subtractive genomics. J Biomol Struct Dyn 40(20):10106–10121

Procter JB, Carstairs GM, Soares B, Mourão K, Ofoegbu TC, Barton D et al (2021) Alignment of biological sequences with Jalview. Springer, US, pp 203–224

Pruitt KD, Tatusova T, Maglott DR (2005) NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res 33(Database issue):D501–4

Quraishi S, Saha D, Kumari K, Jha AN, Roy AS (2024) Non-covalent binding interaction of bioactive coumarin esculetin with calf thymus DNA and yeast transfer RNA: A detailed investigation to decipher the binding affinities, binding location, interacting forces and structural alterations at a molecular level. Int J Biol Macromol 257(Pt 1):128568

Rather MA, Saha D, Bhuyan S, Jha AN, Mandal M (2022) Quorum quenching: a drug discovery approach against *Pseudomonas aeruginosa*. Microbiol Res 264:127173

Saha D, Jha AN (2023) Computational multi-target approach to target essential enzymes of Leishmania donovani using comparative molecular dynamic simulations and MMPBSA analysis. Phytochem Anal 34(7):842–854

Saha D, Borah NJ, Jha AN (2023) Molecular scaffold recognition of drug molecules against essential genes of using biocomputing approach. S Afr J Bot 162:52–63

Sasidharan S, Saudagar P (2021) Leishmaniasis: where are we and where are we heading? Parasitol Res 120(5):1541–1554

Schlein Y (1993) Leishmania and Sandflies: interactions in the life cycle and transmission. Parasitol Today 9(7):255–258

Schmid N, Eichenberger AP, Choutko A, Riniker S, Winger M, Mark AE, Van Gunsteren WF (2011) Definition and testing of the GROMOS force-field versions 54A7 and 54B7. Eur Biophys J 40:843–856

Shahid F, Shehroz M, Zaheer T, Ali A (2020) Subtractive genomics approaches: towards anti-bacterial drug discovery. Front Anti-Infect Drug Discov 8:144–158

Stockdale L, Newton R (2013) A review of preventative methods against human leishmaniasis infection. PLoS Negl Trop Dis 7(6):e2278

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24(8):1596–1599. https://doi.org/10.1093/molbev/msm092

Tashboltayevna AS, Mirzaali o'g'li, Dilshod o'g'li AJOR (2022) Leishmaniosis disease, its symptoms, primary consequences and distribution. Galaxy Int Interdis Res J 10(12):836–838

Tian W, Chen C, Lei X, Zhao J, Liang J (2018) CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res 46(W1):W363-W7

Valdivia HO, Scholte LL, Oliveira G, Gabaldon T, Bartholomeu DC (2015) The Leishmania metaphylome: a comprehensive survey of Leishmania protein phylogenetic relationships. BMC Geno 16:887

Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G et al (2022) AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucl Acids Res 50(D1):D439–D444

Wijnant GJ, Dumetz F, Dirkx L, Bulté D, Cuypers B, Van Bocxlaer K et al (2022) Tackling drug resistance and other causes of treatment failure in Leishmaniasis. Front Trop Dis 3:837460

Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. (2018) DrugBank 5.0: a major update to the DrugBank database for 2018. Nucl Acids Res 46(D1):D1074-D82

World Health Organisation (2022) Available from: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis

Zocher K, Fritz-Wolf K, Kehr S, Fischer M, Rahlfs S, Becker K (2012) Biochemical and structural characterization of Plasmodium falciparum glutamate dehydrogenase 2. Mol Biochem Parasitol 183(1):52–62

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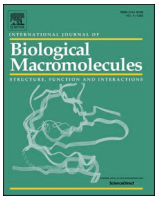

Non-covalent binding interaction of bioactive coumarin esculetin with calf thymus DNA and yeast transfer RNA: A detailed investigation to decipher the binding affinities, binding location, interacting forces and structural alterations at a molecular level

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ABSTRACT

Esculetin is a well-known coumarin derivative found abundantly in nature possessing an extensive array of pharmacological and therapeutic properties. Consequently, to comprehend its molecular recognition mechanism, our objective is to conduct a complete investigation of its interactions with the nucleic acid, specifically ct-DNA, and t-RNA, using spectroscopic and computational techniques. The intrinsic fluorescence of esculetin is quenched when it interacts with ct-DNA and t-RNA, and this occurs through a static quenching mechanism. The thermodynamic parameters demonstrated that the interaction is influenced by hydrogen bonding and weak van der Waals forces. CD and FT-IR results revealed no conformational changes in ct-DNA and t-RNA structure on binding with esculetin. Furthermore, competitive displacement assay with ethidium bromide, melting temperature, viscosity measurement, and potassium iodide quenching experiments, reflected that esculetin probably binds to the minor groove of ct-DNA. The molecular docking results provided further confirmation for the spectroscopic findings, including the binding location of esculetin and binding energies of esculetin complexes with ct-DNA and t-RNA. Molecular dynamics simulation studies demonstrated the conformational stability and flexibility of nucleic acids.

1. Introduction

With the ever-growing number of health problems in the world today, the significance of exploring and creating novel therapeutic substances have become of utmost importance. Natural products sequestered from diverse plants are contributing new advances in medicine owing to their numerous beneficial properties, natural abundance, high stability, low toxicity, relatively low side effects, good biocompatibility, and so on [1]. A category of naturally sourced compounds, primarily obtained from plants, is coumarins, and they possess all the above-mentioned beneficial characteristics. Coumarins are found abundantly in nature and fall under the category of 1,2-benzopyrone derivatives. Their unique and versatile oxygen-containing heterocyclic structure has intrigued the interest of many organic and medicinal chemists [2]. They also have anti-oxidative, anti-cancer, anti-diabetic, anti-coagulant, anti-inflammatory, and anti-viral properties [3–5]. Esculetin (6,7-dihydroxy coumarin), is one of the simplest coumarin derivatives containing two hydroxyl groups at positions C6 and C7 (Fig. 1). It can be extracted from, *Plantago major*, *Aesculus hippocastanum*, *Salvia officinalis*, *Radicula armoracia*, *Ocimum basilicum*, and *Foeniculum vulgare* [6,7]. It is the primary bioactive component of the Chinese herbal medicine *Fraxinus rhynchophylla* [8]. Esculetin has been recognized for its ability to reduce inflammation by obstructing the expression of cytokines related to inflammation [9]. Studies have also shown that esculetin can impede the cell cycle progression and growth of human leukemia HL-60 cells, by causing them to remain in the G1

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absorbance measurements and Stern-Volmer analysis, indicating a static quenching mechanism. The binding affinity (K_b) for esculetin–ct-DNA/t-RNA complexes was found to be moderate, with a value in the order of 10^3 M^{-1}. The thermodynamic analysis of the binding process showed that it occurred spontaneously involving hydrogen bonding as well as van der Waals interaction. CD and FT-IR results conclude that DNA and RNA remain in their original B and A conformational state after binding with esculetin. Competitive displacement assay with EB and KI quenching studies revealed that esculetin binds with DNA via groove mode of binding. The DNA melting study and viscosity measurement provided further evidence to support the groove binding mode of interaction. The impact of ionic strength on the binding process validated the participation of electrostatic interaction in the interaction between esculetin and ct-DNA/t-RNA. Molecular docking was also performed in accordance with our experimental studies, which show that esculetin binds at the guanine, thymine, and adenine base pairs of ct-DNA and the guanine, uracil, and cytosine base pairs of t-RNA. The MD simulation studies revealed that the stability remains same on esculetin binding to ct-DNA and t-RNA. Additionally, it also revealed the type of forces that are involved in the complexation processes between esculetin-ct-DNA/t-RNA. The experimental results were found to be correlating well with the outcomes of the molecular docking and MD simulation studies. Fig. 13 illustrates the several significant findings from the investigation of the interaction between esculetin and ct-DNA/t-RNA. However, our findings provide an important insight into the complexation of esculetin, a compound with great pharmacological properties, with nucleic acids that will assist in the development of a rational drug with improved selectivity and greater clinical efficacy. This study may also contribute to gain insight into the pharmacodynamics of analogous compounds and their derivatives when they interact with nucleic acids.

CRediT authorship contribution statement

Sana Quraishi: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Debanjan Saha:** Investigation, Methodology. **Kalpana Kumari:** Investigation, Methodology. **Anupam Nath Jha:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Atanu Singha Roy:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that no known competing conflict of financial interests or personal relationships exists, that could bias the reported work. All authors have read and appeared in this version of the article.

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Appendix A. Supplementary data

The supplementary Information section contains a full discussion of the instrumentation and methodologies involved in detail along with tables and figures as indicated in different places of the manuscript. Supplementary data to this article can be found online at doi:https://doi.org/10.1016/j.ijbiomac.2023.128568.

References

[1] H. Ji, X. Li, H. Zhang, Natural products and drug discovery, EMBO Rep. 10 (2009) 194–200, https://doi.org/10.1038/embor.2009.12.
[2] M. Lončar, M. Jakovljević, D. Šubarić, M. Pavlić, V. Buzjak Služek, I. Cindrić, M. Molnar, Coumarins in food and methods of their determination, Foods 9 (2020) 645, https://doi.org/10.3390/foods9050645.
[3] F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini, A. Pinto, An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities, Int. J. Mol. Sci. 21 (2020) 4618, https://doi.org/10.3390/ijms21134618.
[4] A. Witaicenis, L.N. Seito, A. da Silveira Chagas, L.D. de Almeida, A.C. Luchini, P. Rodrigues-Orsi, S.H. Cestari, L.C. Di Stasi, Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives, Phytomedicine 21 (2014) 240–246, https://doi.org/10.1016/j.phymed.2013.09.001.
[5] R.N. Gacche, S.G. Jadhav, Antioxidant activities and cytotoxicity of selected coumarin derivatives: preliminary results of a structure–activity relationship study using computational tools, J Exp Clin Med. 4 (2012) 165–169, https://doi.org/10.1016/j.jecm.2012.04.007.
[6] M.J. Matos, L. Santana, E. Uriarte, O.A. Abreu, E. Molina, E.G. Yordi, Coumarins — An Important Class of Phytochemicals, in: Phytochemicals - Isolation, Characterisation and Role in Human Health, InTech, 2015, https://doi.org/10.5772/59982.
[7] Y. Masamoto, H. Ando, Y. Murata, Y. Shimoishi, M. Tada, K. Takahata, Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of Euphorbia lathyris L, Biosci. Biotechnol. Biochem. 67 (2003) 631–634, https://doi.org/10.1271/bbb.67.631.
[8] N.-H. Jeong, E.-J. Yang, M. Jin, J.Y. Lee, Y.-A. Choi, P.-H. Park, S.-R. Lee, S.-U. Kim, T.-Y. Shin, T.K. Kwon, Y.H. Jang, K.-S. Song, S.-H. Kim, Esculetin from Fraxinus rhynchophylla attenuates atopic skin inflammation by inhibiting the expression of inflammatory cytokines, Int. Immunopharmacol. 59 (2018) 209–216, https://doi.org/10.1016/j.intimp.2018.04.005.
[9] Y. Kim, Y. Park, S. Namkoong, J. Lee, Esculetin inhibits the inflammatory response by inducing heme oxygenase-1 in cocultured macrophages and adipocytes, Food Funct. 5 (2014) 2371, https://doi.org/10.1039/C4FO00351A.
[10] C.-Y. Chu, Y.-Y. Tsai, C.-J. Wang, W.-L. Lin, T.-H. Tseng, Induction of apoptosis by esculetin in human leukemia cells, Eur. J. Pharmacol. 416 (2001) 25–32, https://doi.org/10.1016/S0014-2999(01)00859-7.
[11] T. Kaneko, S. Tahara, F. Takabayashi, Suppression of lipid hydroperoxide-induced oxidative damage to cellular DNA by esculetin, Biol. Pharm. Bull. 26 (2003) 840–844, https://doi.org/10.1248/bpb.26.840.
[12] S. Lyndem, R. Gazi, V.K. Belwal, A. Bhatta, M. Jana, A.S. Roy, Binding of bioactive esculin and esculetin with hen egg white lysozyme: spectroscopic and computational methods to comprehensively elucidate the binding affinities, interacting forces, and conformational alterations at molecular level, J. Mol. Liq. 360 (2022), 119423, https://doi.org/10.1016/j.molliq.2022.119423.
[13] S.R. Rajski, B.A. Jackson, J.K. Barton, DNA repair: models for damage and mismatch recognition, mutation research/fundamental and molecular mechanisms of, Mutagenesis 447 (2000) 49–72, https://doi.org/10.1016/S0027-5107(99)00195-5.
[14] M. Airoldi, G. Barone, G. Gennaro, A.M. Giuliani, M. Giustini, Interaction of doxorubicin with polynucleotides, A Spectroscopic Study, Biochemistry. 53 (2014) 2197–2207, https://doi.org/10.1021/bi401687v.
[15] S. Yasmeen, F.A. Qais, M. Rana, A. Islam, Rahisuddin, binding and thermodynamic study of thalidomide with calf thymus DNA: spectroscopic and computational approaches, Int. J. Biol. Macromol. 207 (2022) 644–655, https://doi.org/10.1016/j.ijbiomac.2022.03.036.
[16] Y. Nie, Z. Dai, G. Fozia, J. Zhao, X. Jiang, M. Xu, Y. Ying, Z. Wang, H. Xu Hu, Comparative studies on DNA-binding mechanisms between enantiomers of a polypyridyl ruthenium (II) complex, J. Phys. Chem. B 126 (2022) 4787–4798, https://doi.org/10.1021/acs.jpcb.2c02104.
[17] S. Kumar Tarai, A. Tarai, S. Mandal, B. Nath, I. Som, R. Bhaduri, A. Bagchi, S. Sarkar, A. Biswas, S.Ch. Moi, Cytotoxic behavior and DNA/BSA binding activity of thiosemicarbazone based Ni(II) complex: bio-physical, molecular docking and DFT study, J. Mol. Liq. 383 (2023), 121921, https://doi.org/10.1016/j.molliq.2023.121921.
[18] B. Jana, S. Senapati, D. Ghosh, D. Bose, N. Chattopadhyay, Spectroscopic exploration of mode of binding of ctDNA with 3-hydroxyflavone: a contrast to the mode of binding with flavonoids having additional hydroxyl groups, J. Phys. Chem. B 116 (2012) 639–645, https://doi.org/10.1021/jp2094824.
[19] B. Alberts, A. Johnson, J. Lewis, et al., Molecular Biology of the Cell, 4th edition, Garland Science, New York, 2002. https://www.ncbi.nlm.nih.gov/books/NBK26829/ (accessed March 6, 2023).
[20] H. Kumar, A. Chattopadhyay, R. Prasath, V. Devaraji, R. Joshi, P. Bhavana, P. Saini, S.K. Ghosh, Design, synthesis, physicochemical studies, solvation, and DNA damage of quinoline-appended chalcone derivative: comprehensive spectroscopic approach toward drug discovery, J. Phys. Chem. B 118 (2014) 7257–7266, https://doi.org/10.1021/jp5025262.
[21] T. Nandy, B. Biswas, P.C. Singh, Molecular aspects of the role of groove and stacked regions of DNA in binding with lipids: spectroscopic and docking studies, J. Mol. Liq. 303 (2020), 112672, https://doi.org/10.1016/j.molliq.2020.112672.

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Quorum Quenching: A Drug Discovery Approach Against *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa, a ubiquitous opportunistic and nosocomial biofilm-forming pathogen with complex, interconnected and hierarchical nature of QS systems (*Las*, *Rhl*, *PQS*, and *IQS*), is posing the biggest challenge to the healthcare sector and have made current chemotherapies incapable. Conventional antibiotics designed to intercept the biochemical or physiological processes precisely of planktonic microorganisms exert extreme selective pressure and develop resistance against them thereby emphasizing the development of alternative therapeutic approaches. Additionally, quorum sensing induced pathogenic microbial biofilms and production of virulence factors have intensified the pathogenicity, drug resistance, recurrence of infections, hospital visits, morbidity, and mortality many-folds. In this regard, QS could be a potential druggable target and the discovery of QS inhibiting agents as an anti-virulent measure could serve as an alternative therapeutic approach to conventional antibiotics. Quorum quenching (QQ) is a preferred strategy to combat microbial infections since it attenuates the pathogenicity of microbes and enhances the microbial biofilm susceptibility to antibiotics, thus qualifying as a suitable target for drug discovery. This review discusses the QS-induced pathogenicity of *P. aeruginosa*, the hierarchical QS systems, and QS inhibition as a drug discovery approach to complement classical antibiotic strategy.

1. Introduction

Pseudomonas aeruginosa, a Gram-negative, opportunistic human pathogen, flourishes in diverse environmental niches and nosocomial conditions due to its extraordinary metabolic versatility, genome plasticity, resistance to environmental stresses, intrinsic resistance to antibiotics, strong biofilm-forming potential, and expression of quorum sensing regulated virulence factors (Laborda et al., 2021). *P. aeruginosa* has been reported as one of the major causes of nosocomial infections and a leading pathogen among immunocompromised patients of cystic fibrosis (CT), chronic obstructive pulmonary disease, diffused panbronchiolitis, HIV patients and cancer patients undergoing chemotherapy (Soukarieh et al., 2018; Rather et al., 2021a). *P. aeruginosa* has been regarded as an emerging global public health threat due to its resistance and survival to many available antibiotics and enhanced adaptability and persistence to stressed environmental conditions. It has a high potential of causing life-threatening acute and chronic infections and is the leading cause of morbidity and motility among CT patients (Moradali et al., 2017). *P. aeruginosa* is the most treated infectious pathogen in intensive care units (ICUs) and is persistent in the form of hospital-acquired pneumonia (HAP), urinary tract infections (UTIs), bloodstream infections (BSIs), surgical site infections, central nervous system infections, wound infections, skin and soft tissue infections, bone and joint infections, decubitus ulcers, ocular infections, etc. International Nosocomial Infection Control Consortium (INICC) has described *P. aeruginosa* nosocomial infections as a worldwide healthcare issue and it has been documented that 20% mortality among the patients with *P. aeruginosa* infections has been reported globally while it is 30% and 50% in ventilator-associated pneumonia (VAP) and bacteremia, respectively (Litwin et al., 2021). According to the reports published by

Abbreviations: AIPs, autoinducing peptides; AHLs, acyl-homoserine lactones; AIs, autoinducers; QSSM, quorum sensing signal molecule; QQ, quorum quenching; QQE, quorum quenching enzymes; QSI, quorum sensing inhibitor; HAIs, healthcare-associated infections; CADD, computer-aided drug designing; PDB, protein data bank.

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P. aeruginosa as a model organism that holds promise for the development of potent therapeutic drugs. The anti-QS drugs attenuate the pathogenicity of biofilm-forming bacteria and potentiate biofilm sensitivity to antibiotics, therefore, enhancing antibiotic-mediated biofilm disruption as well. The discovery of QSIs could be an arsenal against biofilm-forming pathogenic microorganisms provided problems associated with them at the cellular and sub-cellular levels are resolved *viz.* target identification, pathogen specificity, drug deliverability, cellular toxicity, etc.

Ethical statement

No human participant and/or animal were used in the study.

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CRediT authorship contribution statement

Muzamil Ahmad Rather did the literature search, data analysis and drafted the review; **Debanjan Saha** drafted in silico section; **Shuvam Bhuyan** and **Muzamil Ahmad Rather** designed the figures; **Anupam Nath Jha:** Writing – review & editing; **Manabendra Mandal:** Funding acquisition, Supervision, Writing – review & editing. All authors contributed to the article and approved the submitted version.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

References

Abbas, H.A., Shaldam, M.A., Eldamasi, D., 2020. Curtailing Quorum Sensing in *Pseudomonas aeruginosa* by Sitagliptin. C. Microbiol 77 (6), 1051–1060. https://doi.org/10.1007/s00284-020-01909-4.

Ahmed, T., Pattnaik, S., Khan, M.B., Ampasala, D.R., Busi, S., Sarma, V.V., 2020. Inhibition of quorum sensing-associated virulence factors and biofilm formation in *Pseudomonas aeruginosa* PAO1 by Mycoleptodiscus indicus PUTY1. B. Jor. Microbiol 51 (2), 467–487. https://doi.org/10.1007/s42770-020-00235-y.

Alcalde-Rico, M., Hernando-Amado, S., Blanco, P., Martinez, J.L., 2016. Multidrug Efflux Pumps at the Crossroad between Antibiotic Resistance and Bacterial Virulence. F. Microbiol. 7, 1483. https://doi.org/10.3389/fmicb.2016.01483.

Al-Shabib, N.A., Husain, F.M., Rehman, M.T., Alyousef, A.A., Arshad, M., Khan, A., Masood Khan, J., Alam, P., Albalawi, T.A., Shahzad, S.A., Syed, J.B., Al-Ajmi, M.F., 2020. Food color 'Azorubine' interferes with quorum sensing regulated functions and obliterates biofilm formed by food associated bacteria: an in vitro and in silico approach. S. J. Biol. Scien 27 (4), 1080–1090. https://doi.org/10.1016/j.sjbs.2020.01.001.

Anandan, K., Vittal, R.R., 2019. Quorum quenching activity of AiiA lactonase KMMI17 from endophytic *Bacillus thuringiensis* KMCL07 on AHL- mediated pathogenic phenotype in *Pseudomonas aeruginosa*. M. Pathog. 132, 230–242. https://doi.org/10.1016/j.micpath.2019.05.015.

Anju, V.T., Paramanantham, P., SB, S.L., Sharan, A., Syed, A., Bahkali, N.A., Alsaedi, M. H., Kaviyarasu, K., Busi, S., 2019a. Antimicrobial photodynamic activity of toluidine blue-carbon nanotube conjugate against Pseudomonas aeruginosa and Staphylococcus aureus-understanding the mechanism of action. Photo Photodyn. 27, 305–316. https://doi.org/10.1016/j.pdpdt.2019.06.014.

Anju, V.T., Paramanantham, P., Siddhardha, B., Lal, S.S., Sharan, A., Alyousef, A.A., Arshad, M., Syed, A., 2019b. Malachite green-conjugated multi-walled carbon nanotubes potentiate antimicrobial photodynamic inactivation of planktonic cells and biofilms of Pseudomonas aeruginosa and Staphylococcus aureus. Int. J. Nanomed. 14, 3861 https://doi.org/10.2147%2FIJN.S202734.

Aswathanarayan, J.B., Vittal, R.R., 2018. Inhibition of biofilm formation and quorum sensing mediated phenotypes by berberine in *Pseudomonas aeruginosa* and *Salmonella typhimurium*. Rsc. Adv. 8, 36133–36141. https://doi.org/10.1039/C8RA06413J.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., International Natural Product Sciences, T., Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. N. R. Dru. Disco 20 (3), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Banerjee, G., Ray, A.K., 2016. The talking language in some major Gram-negative bacteria. A. Microbiol. 198 (6), 489–499. https://doi.org/10.1007/s00203-016-1220-x.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The Protein Data Bank. N. Acids Res. 28 (1), 235–242. https://doi.org/10.1093/nar/28.1.235.

Brindhadevi, K., LewisOscar, F., Mylonakis, E., Shanmugam, S., Verma, T.N., Pugazhendhi, A., 2020. Biofilm and Quorum sensing mediated pathogenicity in *Pseudomonas aeruginosa*. P. Biochem 96, 49–57. https://doi.org/10.1016/j.procbio.2020.06.001.

Bzdrenga, J., Daude, D., Remy, B., Jacquet, P., Plener, L., Elias, M., Chabriere, E., 2017. Biotechnological applications of quorum quenching enzymes. C. Biol. Interact. 267, 104–115. https://doi.org/10.1016/j.cbi.2016.05.028.

Chatterjee, M., D'Morris, S., Paul, V., Warrier, S., Vasudevan, A.K., Vanuopadath, M., Nair, S.S., Paul-Prasanth, B., Mohan, C.G., Biswas, R., 2017. Mechanistic understanding of Phenyllactic acid mediated inhibition of quorum sensing and biofilm development in *Pseudomonas aeruginosa*. A. Micro Biotechnol. 101 (22), 8223–8236. https://doi.org/10.1007/s00253-017-8546-4.

Chen, X., Yu, F., Li, Y., Lou, Z., Toure, S.L., Wang, H., 2020. The inhibitory activity of p-coumaric acid on quorum sensing and its enhancement effect on meat preservation. CyTA – Jour. Fo. 18 (1), 61–67. https://doi.org/10.1080/19476337.2019.1701558.

Cheng, W.J., Zhou, J.W., Zhang, P.P., Luo, H.Z., Tang, S., Li, J.J., Deng, S.M., Jia, A.Q., 2020. Quorum sensing inhibition and tobramycin acceleration in *Chromobacterium violaceum* by two natural cinnamic acid derivatives. A. Microbiol. Biotechnol. 104 (11), 5025–5037. https://doi.org/10.1007/s00253-020-10593-0.

Control ECfDPa, 2018. Healthcare-associated infections acquired in intensive care units. In: ECDC Annual epidemiological report for 2016, Available at https://www.ecdc.europa.eu/en/publications-data/healthcare-associated-infections-intensive-care-units-annual-epidemiological-0[Verified (Accessed 18 March 2022).

Das, A., Das, M.C., Sandhu, P., Das, N., Tribedi, P., De, U.C., Akhter, Y., Bhattacharjee, S., 2017. Antibiofilm activity of *Parkia javanica* against *Pseudomonas aeruginosa*: a study with fruit extract. Rsc. Adv. 8, 5497–5513. https://doi.org/10.1039/C6RA24603F.

Das, M.C., Sandhu, P., Gupta, P., Rudrapaul, P., De, U.C., Tribedi, P., Akhter, Y., Bhattacharjee, S., 2016. Attenuation of Pseudomonas aeruginosa biofilm formation by Vitexin: a combinatorial study with azithromycin and gentamicin. Sci. Repo. 6, 23347. https://doi.org/10.1038/srep23347.

Du, Y., Sun, J., Gong, Q., Wang, Y., Fu, P., Zhu, W., 2018. New alpha-pyridones with quorum-sensing inhibitory activity from diversity-enhanced extracts of a streptomyces sp. derived from marine algae. J. Agric. Fo. Chem. 66 (8), 1807–1812. https://doi.org/10.1021/acs.jafc.7b05330.

Fong, J., Zhang, C., Yang, R., Boo, Z.Z., Tan, S.K., Nielsen, T.E., Givskov, M., Liu, X.W., Bin, W., Su, H., Yang, L., 2018. Combination therapy strategy of quorum quenching enzyme and quorum sensing inhibitor in suppressing multiple quorum sensing pathways of *P. aeruginosa*. Sci. Rep. 8 (1), 1155. https://doi.org/10.1038/s41598-018-19504-w.

Ghoreishi, F.S., Roghanian, R., Emtiazi, G., 2020. Inhibition of quorum sensing-controlled virulence factors with natural substances and novel protease, obtained from Halobacillus karajensis. Micro Pathog. 149, 104555 https://doi.org/10.1016/j.micpath.2020.104555.

Gokalsin, B., Aksoydan, B., Erman, B., Sesal, N.C., 2017. Reducing virulence and biofilm of pseudomonas aeruginosa by potential quorum sensing inhibitor carotenoid: zeaxanthin. Micro Ecol. 74 (2), 466–473. https://doi.org/10.1007/s00248-017-0949-3.

Hernando-Amado, S., Alcalde-Rico, M., Gil-Gil, T., Valverde, J.R., Martinez, J.L., 2020. Naringenin Inhibition of the *Pseudomonas aeruginosa* quorum sensing response is based on its time-dependent competition With N-(3-Oxo-dodecanoyl)-L-homoserine Lactone for LasR Binding. F. Mol. Biosci. 7, 25. https://doi.org/10.3389/fmolb.2020.00025.

Jensen, V., Lons, D., Zaoui, C., Bredenbruch, F., Meissner, A., Dieterich, G., Munch, R., Haussler, S., 2006. RhlR expression in *Pseudomonas aeruginosa* is modulated by the *Pseudomonas* quinolone signal via PhoB-dependent and -independent pathways. J. Bacteriol. 188 (24), 8601–8606. https://doi.org/10.1128/JB.01378-06.

Kalia, V.C., Patel, S.K.S., Kang, Y.C., Lee, J.K., 2019. Quorum sensing inhibitors as antipathogens: biotechnological applications. Biot. Advan. 37 (1), 68–90. https://doi.org/10.1016/j.biotechadv.2018.11.006.

Kim, H.S., Lee, S.H., Byun, Y., Park, H.D., 2015. 6-Gingerol reduces *Pseudomonas aeruginosa* biofilm formation and virulence via quorum sensing inhibition. Sci. Rep. 5, 8656. https://doi.org/10.1038/srep08656.

Laborda, P., Sanz-Garcia, F., Hernando-Amado, S., Martinez, J.L., 2021. *Pseudomonas aeruginosa*: an antibiotic resilient pathogen with environmental origin. C. Opin. Microbiol. 64, 125–132. https://doi.org/10.1016/j.mib.2021.09.010.

Lee, J., Zhang, L., 2015. The hierarchy quorum sensing network in Pseudomonas aeruginosa. Prot. Cel. 6 (1), 26–41. https://doi.org/10.1007/s13238-014-0100-x.

Lee, J., Wu, J., Deng, Y., Wang, J., Wang, C., Wang, J., Chang, C., Dong, Y., Williams, P., Zhang, L.H., 2013. A cell-cell communication signal integrates quorum sensing and stress response. N. Chem. Biol. 9 (5), 339–343. https://doi.org/10.1038/nchembio.1225.

Li, X., Sun, L., Zhang, P., Wang, Y., 2021. Novel approaches to combat medical device-associated biofilms. Coatings 11 (3), 294. https://doi.org/10.3390/coatings11030294.

Lidor, O., Al-Quntar, A., Pesci, E.C., Steinberg, D., 2015. Mechanistic analysis of a synthetic inhibitor of the *Pseudomonas aeruginosa* LasI quorum-sensing signal synthase. Sci. Rep. 5, 16569. https://doi.org/10.1038/srep16569.

CHAPTER

Review of methods for encapsulation of nutraceutical compounds

5

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1. Introduction

Nutraceuticals are an increasingly researched area in the pharmaceutical and food industries, and their functions have piqued people's attention. Public awareness about general health has spawned a thriving sector focusing on food-derived bioactive substances with disease-preventing properties, known as nutraceuticals. Consumers are becoming increasingly concerned about food's nutritional elements, and the latest advances in the field of study of functional food reflect this reality. In the development of unique goods, a better balance between nutrients, the addition of indigestible fractions, and the complementation of trace elements, vitamins, or specific components appear to be necessary. Furthermore, the growing recognition of the role of nutraceuticals like probiotics in enhancing human health has sparked a fascination in developing novel forms of administration methods that encapsulate and protect probiotics so that they can be delivered successfully to the target area [1].

Nutraceuticals have garnered substantial attention recently due to their health benefits and disease-prevention properties. The growing number of commercially available nutraceuticals and their diverse range of applications reflect the global predominance of nutraceuticals. As a result, a unique opportunity to generate next-generation nutraceuticals using novel, dependable, low-cost methodologies has emerged [2]. Several nutraceuticals have been identified as promising agents for the prevention and treatment of a variety of diseases, including allergies, cancer, cardiovascular and ocular disorders, and Parkinson's disease, including the regulation of immune system functionality and inflammation, according to recent research. As a result, nutraceuticals have gained much attention, which opens up new possibilities for development of unique products intended in satisfying customer demand for health-enhancing meals [3].

Nutraceuticals or functional foods are divided into at least two groups. The first category comprises foods that are naturally high in nutraceutical components. In contrast, the second category includes foods that have been produced with effective

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the source of bioactive compounds, material used to be encapsulated, and the specific target where it is going to be delivered. Research is carried out to improve and boost the techniques to make the encapsulation scientifically more stable along with being economically feasible for all types of consumers.

Regardless of its application in the field of the food industry, encapsulation of nutraceuticals does not avail to attract the consumers. This drawback is mainly because of the effectiveness, cost, and lack of awareness. Moreover, people are more used to taking chemically synthesized medicines than nutraceuticals as therapeutic drugs. To maximize the potential of nutraceuticals, better encapsulation techniques that can scale up the process and cost-effectivity and can attract the consumers are needed. In addition, suitable materials, odorless food, fewer side effects, high shelf-life, and safety and trade regulations can uplift the valuation. In the future, advanced encapsulation technological techniques will come up, which will revolutionize the therapeutic scenario.

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References

[1] M.F. Yao, et al., Progress in microencapsulation of probiotics: a review, Compr. Rev. Food Sci. Food Saf. 19 (2) (2020) 857–874.
[2] P.M. Reque, A. Brandelli, Encapsulation of probiotics and nutraceuticals: applications in functional food industry, Trends Food Sci. Technol. 114 (2021) 1–10.
[3] T. Tsiaka, et al., Design and development of novel nutraceuticals: current trends and methodologies, Nutraceuticals 2 (2) (2022) 71–90.
[4] A. Santini, et al., Nutraceuticals: opening the debate for a regulatory framework, Br. J. Clin. Pharmacol. 84 (4) (2018) 659–672.
[5] S.L. Defelice, The nutraceutical revolution—its impact on food-industry R-and-D, Trends Food Sci. Technol. 6 (2) (1995) 59–61.
[6] S.H. Zeisel, Regulation of "nutraceuticals", Science 285 (5435) (1999) 1853–1855.
[7] I.G. Telessy, Nutraceuticals, in: The role of Functional Food Security in Global Health, 2019, pp. 409–421.
[8] A.A.A. El-kader, H.M.A. Hashish, Encapsulation techniques of food bioproduct, Egypt. J. Chem. (2019).
[9] J.W. Chen, L.D. Hu, Nanoscale delivery system for nutraceuticals: preparation, application, characterization, safety, and future trends, Food Eng. Rev. 12 (1) (2020) 14–31.
[10] M.M. Leena, et al., Nanoencapsulation of nutraceutical ingredients, in: Biopolymer-Based Formulations, 2020, pp. 311–352.
[11] D. Jones, S. Caballero, G. Davidov-Pardo, Bioavailability of nanotechnology-based bioactives and nutraceuticals, Adv. Food Nutr. Res. 88 (2019) 235–273.