Vol. 7, No. 3, December 2025, pp xxx-xxx
Crossref https://doi.org/10.36590/jika.v7i3.1465
http://salnesia.id/index.php/jika
jika@salnesia.id, p-ISSN: 2337-9847, e-ISSN: 2686-2883
Penerbit: Sarana Ilmu Indonesia (salnesia)

RESEARCH ARTICLES

Analisis Bioinformatika Target Potensial Asam Betulinat terhadap Faktor Virulensi *Chlamydia trachomatis*

Bioinformatics Analysis of Potential Targets of Betulinic Acid Against Virulence Factors of Chlamydia trachomatis

Erla Nurani[1], Putri Diana Roza[1], Darmawi Darmawi[2], Lalu Muhammad Irham[3], Wirawan Adikusuma[4], Dewi Anggraini[5*]

[1]Program in Biomedical Sciences, Faculty of Medicine, Postgraduate, Universitas Riau, Pekanbaru, Indonesia
[2]Department of Histology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia.
[3]Department of Pharmacology and Clinical Pharmachy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[4]Department of Pharmacy, University of Muhammadiyah Mataram, Mataram, Indonesia
[5]Department of Microbiology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia

Abstract

Chlamydia trachomatis is an obligate intracellular pathogen responsible for trachoma and sexually transmitted infections, with rising antibiotic resistance posing a major therapeutic challenge. Natural compounds such as betulinic acid, a pentacyclic triterpenoid with broad-spectrum antimicrobial and anti-inflammatory properties, offer potential as alternative therapeutic agents. This study aimed to analyze the potential targets of betulinic acid against C. trachomatis virulence factors using bioinformatics approaches. Protein–compound interaction prediction was performed using STITCH v5.0, while virulence classification was analyzed through VICMpred and VirulentPred. BepiPred v2.0 was employed to identify B-cell epitopes, and PSORTb v3.0 was used to predict subcellular localization. The results identified five proteins targeted by betulinic acid, including DNA topoisomerase IV subunit B (parE), DNA topoisomerase IV subunit A (parC), DNA gyrase subunits (gyrA and gyrB), and sulfite reductase (cysJ). Among these, three were classified as virulence factors: parE (0,2959), parC (0,1754), and cysJ (0,4018). Subcellular localization analysis showed that parE and parC are cytoplasmic proteins essential for DNA replication, while cysJ is associated with the cytoplasmic membrane and metabolic processes. Betulinic acid showed strong potential as an antimicrobial compound targeting key virulence proteins of C. trachomatis. These findings provide foundational insight for further experimental studies to develop betulinic acid–based therapeutic strategies against chlamydial infections.

Keywords: *Betulinic acid, bioinformatics, Chlamydia trachomatis*

Article history :

Submitted 31 January 2025
Accepted 30 December 2025
Published 31 December 2025

PUBLISHED BY:
Sarana Ilmu Indonesia (salnesia)
Address:
Jl. Dr. Ratulangi No. 75A, Baju Bodoa, Maros Baru, Kab. Maros, Provinsi Sulawesi Selatan, Indonesia

Email:
info@salnesia.id, jika@salnesia.id
Phone:
+62 85255155883

Abstrak

Chlamydia trachomatis adalah patogen intraseluler obligat yang menyebabkan trakoma dan infeksi menular seksual, dengan meningkatnya resistensi antibiotik yang menimbulkan tantangan terapeutik yang besar. Senyawa alami seperti asam betulinat, suatu triterpenoid pentasiklik dengan sifat antimikroba dan antiinflamasi spektrum luas, menawarkan potensi sebagai agen terapeutik alternatif. Penelitian ini bertujuan untuk menganalisis target potensial asam betulinat terhadap faktor virulensi *C. trachomatis* menggunakan pendekatan bioinformatika. Prediksi interaksi protein-senyawa dilakukan menggunakan STITCH v5.0, sementara klasifikasi virulensi dianalisis menggunakan VICMpred dan VirulentPred. BepiPred v2.0 digunakan untuk mengidentifikasi epitop sel B, dan PSORTb v3.0 digunakan untuk memprediksi lokalisasi subseluler. Hasil penelitian mengidentifikasi lima protein yang ditargetkan oleh asam betulinat, meliputi subunit DNA topoisomerase IV B (parE), subunit DNA topoisomerase IV A (parC), subunit DNA girase (gyrA dan gyrB), dan reduktase sulfit (cysJ). Di antara kelima protein tersebut, tiga di antaranya diklasifikasikan sebagai faktor virulen: parE (0,2959), parC (0,1754), dan cysJ (0,4018). Analisis lokalisasi subseluler menunjukkan bahwa parE dan parC merupakan protein sitoplasmik yang esensial untuk replikasi DNA, sementara cysJ berkaitan dengan membran sitoplasma dan proses metabolisme. Asam betulinat menunjukkan potensi yang kuat sebagai senyawa antimikroba yang menargetkan protein virulensi kunci *C. trachomatis*. Temuan ini memberikan wawasan dasar untuk studi eksperimental lebih lanjut guna mengembangkan strategi terapi berbasis asam betulinat untuk melawan infeksi klamidia.

Kata Kunci: *asam Betulinat,* bioinformatika, *Chlamydia trachomatis*

*Correspondence Author:
Dewi Anggraini, email: dewianggrainiyovi@gmail.com

Highlight:

- Betulinic acid is identified as a promising natural therapeutic agent against *Chlamydia trachomatis* due to its ability to target key proteins involved in the bacteria's survival and infection process.
- Through bioinformatics analysis (STITCH v5.0 and VICMpred), the study identified specific high-confidence target proteins, such as DnaK and FabI, which are essential for the metabolic and functional virulence of the pathogen.
- The interaction between betulinic acid and these protein targets suggests a mechanism that can disrupt the bacteria's ability to replicate and maintain its structure, offering a potential alternative to overcome antibiotic resistance.

INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes trachoma and sexually transmitted infections (STIs), affecting millions of people worldwide. In 2020, the World Health Organization reported an estimated 128.5 million new *C. trachomatis* infections among adults aged 15–49 years, with a global prevalence of 4.0% in women and 2.5% in men, making it one of the most common bacterial STIs worldwide. In developing countries, trachoma remains a leading cause of preventable blindness, while untreated genital infections can result in severe complications, including infertility and pelvic inflammatory disease (WHO, 2025). The current standard treatment

for *C. trachomatis* infections primarily relies on antibiotics, with doxycycline serving as one of the first-line therapies (Rodrigues et al., 2022). Although *C. trachomatis* is generally susceptible to antibiotics that interfere with DNA and protein synthesis such as tetracyclines, macrolides, fluoroquinolones, rifamycins, and lincosamides the effectiveness of these treatments is increasingly challenged by the emergence of antibiotic resistance (Benamri et al., 2021). This growing concern has intensified the search for alternative therapeutic agents, particularly those derived from natural sources (Rodrigues et al., 2022).

Betulinic acid, a naturally occurring pentacyclic triterpenoid, has shown promising antimicrobial and anti-inflammatory properties in various studies (Oliveira-Costa et al., 2022). Its broad spectrum of biological activities, including antibacterial, antiviral, and anti-inflammatory effects, makes it an attractive candidate for therapeutic development (Lou et al., 2021). However, its potential interaction with *C. trachomatis* virulence factors and the underlying molecular mechanisms remain unexplored.

Bioinformatics approaches offer powerful tools for predicting drug-protein interactions and understanding molecular mechanisms of drug action (Xia, 2017). This study utilizes various bioinformatics tools including STITCH v5.0 for protein-drug and interaction prediction, VICMPred and VirulentPred for protein function and virulence classification, BepiPred v2.0 for B-cell epitope identification, and PSORTb v3.0 for subcellular localization prediction (Devi and Priyadharsini, 2022). This comprehensive analysis aims to identify potential virulence factors of *C. trachomatis* that could be targeted by betulinic acid.

The primary objective of this research is to predict and analyze the interactions between betulinic acid and *C. trachomatis* virulence factors using bioinformatics approaches. Understanding these molecular interactions could provide valuable insights for developing more effective therapeutic strategies against chlamydial infections. This study represents the first comprehensive bioinformatics analysis of betulinic acid's potential targets among *C. trachomatis* virulence factors, offering new perspectives for treating chlamydial infections.

METHODS

This study utilized various bioinformatics tools to explore the interactions between betulinic acid and the virulence factors of *Chlamydia trachomatis*. The primary objective of this research was to identify potential protein targets and assess their biological significance in the context of virulence and pathogenicity. This study was conducted using a computational approach with several interconnected key steps.

Protein-compound interaction prediction was performed using STITCH v5.0 (http://stitch.embl.de/, accessed on November 15, 2024), a freely accessible web-based tool. The process began by inputting betulinic acid as the query molecule using its PubChem CID, then selecting *Chlamydia trachomatis* as the target organism with Taxonomic ID 813. Subsequently, detected interactions were filtered and annotated based on interaction types, including binding, activation, or inhibition (Szklarczyk et al., 2016).

Protein sequences of the identified virulence factors of *Chlamydia trachomatis* were retrieved from the NCBI database in FASTA format for further analysis. The steps involved downloading protein sequences in FASTA format, verifying sequence integrity, and ensuring compatibility with subsequent prediction tools.

Virulence factors were predicted using VICMPred via the web-based VICMpred platform (https://webs.iiitd.edu.in/, accessed on November 15, 2024). The analysis

included uploading protein sequences in FASTA format, evaluating the virulence potential based on predictive algorithms, and selecting proteins with high virulence scores for further investigation. To confirm these predictions, an additional analysis was performed using VirulentPred (https://bioinfo.icgeb.res.in/, accessed on November 15, 2024), which classified the retrieved protein sequences into virulent and non-virulent categories. This process involved inputting protein sequences in FASTA format, analyzing prediction scores with a virulence threshold of >0.5, and confirming virulence-associated proteins relevant to betulinic acid interactions (Praja and Rosalina, 2022).

To identify epitopes within the determined virulence factors, an analysis was conducted using BepiPred v2.0 (http://tools.immuneepitope.org/bcell/, accessed on November 15, 2024). Prediction was performed by inputting FASTA sequences of virulence proteins, identifying potential epitopes with high antigenicity scores, and mapping these epitopes to regions potentially targeted by betulinic acid to understand its immune-modulatory effects (Jespersen et al., 2017).

Finally, subcellular localization prediction of the identified virulence factors was carried out using PSORTb v3.0 (https://www.psort.org/psortb/, accessed on November 15, 2024) to determine their functional roles. Identified FASTA protein sequences were uploaded into this software to predict localization categories such as cytoplasmic, membrane-bound, or extracellular. Proteins localized in the membrane or extracellular space were prioritized as potential targets for antimicrobial therapy development based on betulinic acid (Praja and Rosalina, 2022).

RESULTS AND DISCUSSIONS

STITCH v5.0

Bioinformatics analysis using STITCH v5.0 identified five proteins of *C. trachomatis* targeted by betulinic acid Figure 1. These proteins were parE, parC, gyrB, gyrA, and cysJ, shown as identifier codes with distinct protein names, functions, and properties. Four proteins targeted by betulinic acid (parE, parC, gyrB and gyrA) demonstrated interactions with each other.

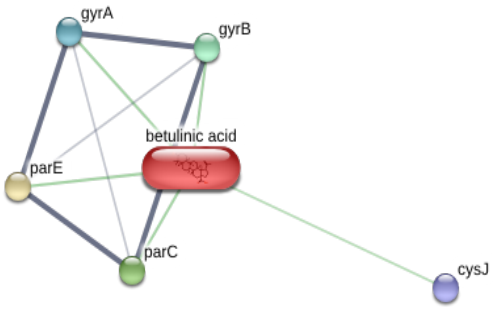

Figure 1. Results of the interaction analysis of *Chlamydia trachomatis* with betulinic acid

VICMPred and VirulentPred

Betulinic acid was found to interact with various proteins in *C. trachomatis* Table 1. The five identified proteins were parE, parC, gyrB, gyrA, and cysJ. These proteins were associated with cellular processes, while parC was also involved in *C. trachomatis* metabolism. VirulentPred analysis of *C. trachomatis* proteins identified

three key virulence factors, DNA topoisomerase IV subunit B (parE) with a VirulentPred score of 0,2959, DNA topoisomerase IV subunit A (parC) with a score of 0.1754, and Sulfite Reductase (cysJ) with a score of 0.4018.

Table 1. Results of functional class, virulence property analysis and subcellular location of *Chlamydia trachomatis* with betulinic acid

Compound	Identifier	Protein	VICMPred Functional Class	Virulent/Non virulent	VirulentPred Score
	parE	*DNA topoisomerase IV subunit B (605 aa)*	Cellular Process	Virulent	0,2959
Betulinic acid	parC	*DNA topoisomerase IV subunit A (490 aa)*	Cellular Process and Metabolism	Virulent	0,1754
	gyrB	*DNA gyrase subunit B (804 aa)*	Cellular Process	Non-Virulent	-1,274
	gyrA	*DNA gyrase subunit A (836 aa)*	Cellular Process	Non-Virulent	-1,165
	cysJ	*Sulfite Reductase (350 aa)*	Cellular Process	Virulent	0,4018

BepiPred v2.0

The three important virulent proteins of *C. trachomatis* were then predicted for their epitope sites using BepiPred v2.0. The epitope prediction results showed that DNA topoisomerase IV subunit B (parE), DNA topoisomerase IV subunit A (parC), Sulfite Reductase (cysJ) proteins had 20, 18 and 19 epitope sites, respectively Figure 2. These results provide a foundational basis for experimental validation to confirm the immunogenicity and functionality of these predicted peptides. The identification of such epitopes is essential for developing vaccines, diagnostic tools, or targeted therapies against the associated pathogen.

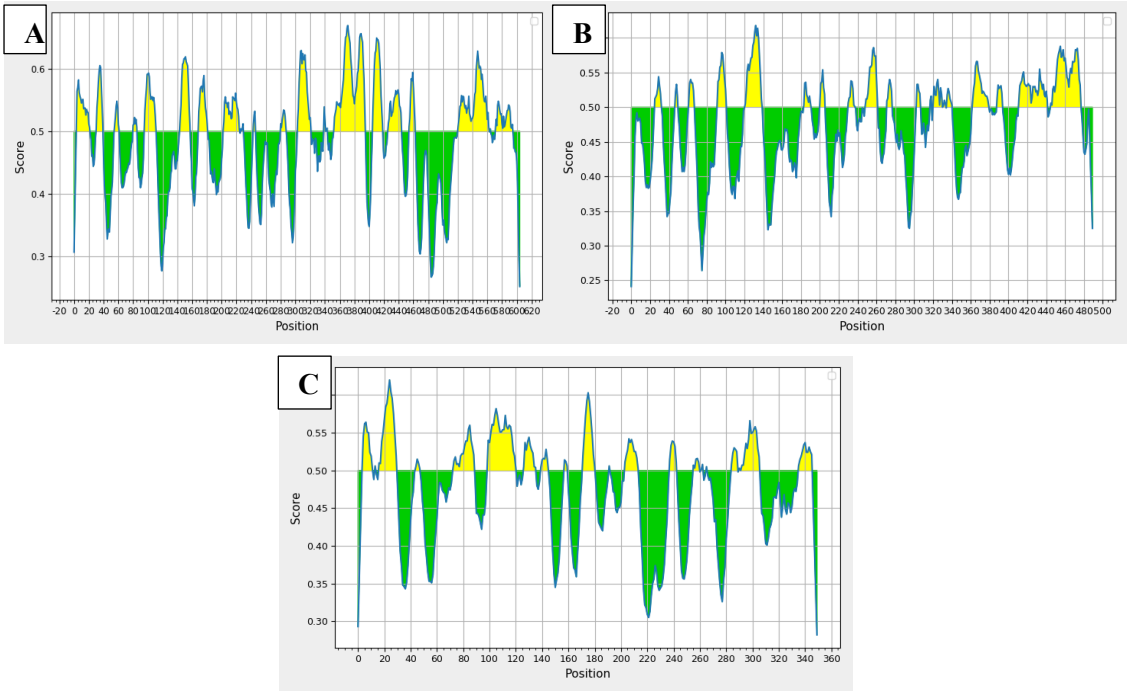

Figure 2. Predicted epitopes on the virulence protein (A) DNA topoisomerase IV subunit B (parE), (B) DNA topoisomerase IV subunit A (parC), (C) sulfite reductase (cysJ)

PSORTb v3.0

The analysis identified five potential target proteins of betulinic acid in *Chlamydia trachomatis*, with their subcellular localizations predicted using PSORTb v3.0 Table 2. Four of the proteins, including DNA topoisomerase IV subunit B (parE, 605 amino acids), DNA topoisomerase IV subunit A (parC, 490 amino acids), DNA gyrase subunit B (gyrB, 804 amino acids), and DNA gyrase subunit A (gyrA, 836 amino acids), were localized in the cytoplasm. These proteins are essential for DNA replication and repair, making them critical targets for antimicrobial intervention. The fifth protein, sulfite reductase (cysJ, 350 amino acids), was associated with the cytoplasmic membrane, indicating its potential role in metabolic processes. The subcellular localization of *C. trachomatis* virulence proteins was predicted using PSORTb v3.0. To ensure accuracy, each prediction was confirmed by comparing the results with functional annotations available in the UniProt database and related literature. The localization of cysJ to the cytoplasmic membrane was consistent with its enzymatic role in sulfur metabolism, which typically occurs at the membrane cytoplasm interface. These results suggest that betulinic acid may exert its antimicrobial activity by interacting with key proteins involved in vital cellular processes, providing a promising foundation for developing novel therapeutic strategies against *C. trachomatis* infections.

Chlamydia trachomatis is a significant human pathogen responsible for a wide range of diseases, including trachoma and sexually transmitted infections (STIs) (Witkin et al., 2017). Its ability to survive and replicate within host cells is driven by various virulence factors that facilitate adhesion, invasion, immune evasion, and intracellular persistence (Jury et al., 2023). Understanding the molecular mechanisms behind these processes is crucial for developing effective treatments, especially in light of increasing antibiotic resistance. This study presents a comprehensive bioinformatics analysis to

explore the potential interactions of betulinic acid with the virulence factors of *Chlamydia trachomatis*. The findings identified five key proteins, primarily involved in DNA replication and metabolic processes, as potential targets.

Table 2. Results of the subcellular localization of *C. trachomatis* virulence factors with betulinic acid

Compound	Identifier	Subcellular Location PSORTb v3.0
Betulinic acid	parE	Cytoplasmic
	parC	Cytoplasmic
	gyrB	Cytoplasmic
	gyrA	Cytoplasmic
	cysJ	Cytoplasmic Membrane

This study identified three key virulence proteins of *C. trachomatis* that potentially interact with betulinic acid: DNA topoisomerase IV subunit B (parE), DNA topoisomerase IV subunit A (parC), and Sulfite Reductase (cysJ). By targeting these proteins, betulinic acid could disrupt these essential functions, leading to bacterial cell death (Rodrigues et al., 2023). The interactions between betulinic acid and these proteins suggest potential mechanisms for its antimicrobial activity. ParE and parC, located in the cytoplasm, are essential for DNA replication and cell division in bacteria, making them crucial targets for antimicrobial compounds (Orillard and Tan, 2016). The cytoplasmic membrane location of cysJ indicates its accessibility to external compounds, potentially facilitating interaction with betulinic acid. This strategic distribution of target proteins across different cellular compartments indicates multiple potential mechanisms of action for betulinic acid against *C. trachomatis*.

Proteins like cysJ often contain signal peptides that guide their localization within cellular compartments. These peptides ensure that proteins reach their intended destinations, such as the cytoplasmic membrane, where they can interact with external substances. The molecular mechanisms underlying these interactions are supported by the VirulentPred analysis scores, with cysJ showing the highest virulence prediction score (0.4018), followed by parE (0,2959) and parC (0,1754). The simultaneous targeting of both topoisomerase IV subunits (parE and parC) is particularly noteworthy, as it suggests a potential dual-inhibition mechanism that could enhance antimicrobial efficacy (Kokot et al., 2022). This dual-targeting approach could be especially valuable in preventing the development of drug resistance, as mutations would need to occur in multiple targets simultaneously to confer resistance. Additionally, the interaction with cysJ, involved in sulfur metabolism, suggests that betulinic acid may also interfere with essential metabolic pathways in *C. trachomatis* (Adepoju et al., 2023).

Topoisomerase IV is primarily involved in decatenating daughter chromosomes during replication, while DNA gyrase introduces negative supercoils into DNA, facilitating replication and transcription processes. By targeting both enzymes, compounds can disrupt these critical processes more effectively than those targeting a single enzyme (D'Atanasio et al., 2020). The dual-targeting approach significantly lowers the likelihood of resistance development. Research indicates that when both topoisomerase IV and gyrase are inhibited, the frequency of selecting resistant mutants is markedly reduced compared to treatments with single-target inhibitors. This is because any potential resistance would necessitate simultaneous mutations in both enzymes, a rare occurrence in bacterial populations (Bisacchi and Manchester, 2015).

The epitope analysis revealed multiple immunogenic regions in these proteins, particularly notable in parC (positions 122-139) and parE (positions 405-422) with high prediction scores. These findings suggest that betulinic acid might interfere with critical protein functions by targeting these epitope-rich regions. The presence of multiple epitope sites, especially in membrane-associated proteins like cysJ, could enhance the compound's ability to disrupt bacterial cellular processes. The ability of betulinic acid to inhibit DNA topoisomerases suggests its potential as an antibacterial agent (Rodrigues et al., 2023). This multifaceted approach not only compromises DNA replication but may also interfere with vital metabolic functions, further enhancing antimicrobial efficacy. In summary, the dual targeting of topoisomerase IV subunits presents a strategic advantage in combating bacterial infections by enhancing antimicrobial action and mitigating the risk of developing drug-resistant strains. The exploration of such compounds could lead to significant advancements in antibiotic therapy (Grygiel-Górniak and Folga, 2023).

This report is supported by a study conducted by Ganguly *et al.* Betulinic acid has been identified as a catalytic inhibitor of topoisomerases, particularly topoisomerase I, although its effects on topoisomerase IV are also significant. It disrupts the formation of topoisomerase–DNA complexes, which are essential for the enzyme's role in DNA replication and repair processes. Such inhibition can lead to increased cellular stress and induce apoptosis in bacterial cells (Grossman et al., 2023).

The clinical relevance of these findings extends beyond theoretical interactions, offering promising insights for alternative therapeutic development against *C. trachomatis* infections. While current treatments rely heavily on conventional antibiotics such as doxycycline and macrolides, betulinic acid's multiple protein targets suggest a novel therapeutic approach that could potentially overcome existing antibiotic resistance mechanisms (Rodrigues et al., 2023). The compound's natural origin and established safety profile in previous studies further enhance its therapeutic potential, particularly in an era of increasing antimicrobial resistance (Lou et al., 2021).

This study, however, has several limitations that warrant consideration. As an in silico investigation, our findings represent theoretical predictions that require experimental validation through biochemical and cellular assays. The bioinformatics tools used, while powerful, have inherent limitations in their predictive capabilities and may not fully capture the complexity of protein-ligand interactions in physiological conditions. Additionally, factors such as drug bioavailability, cellular penetration, and potential off-target effects cannot be fully assessed through computational analysis alone.

Future research directions should focus on experimental validation of these predicted interactions, particularly through in vitro binding studies and antimicrobial susceptibility testing. Investigation of betulinic acid derivatives could optimize their interaction with the identified target proteins, potentially enhancing therapeutic efficacy. The methodological framework established in this study could also be applied to screen other natural compounds against *C. trachomatis* virulence factors, potentially identifying additional therapeutic candidates. Furthermore, understanding the structure-activity relationships between betulinic acid and its bacterial targets could guide rational drug design efforts, leading to more effective treatments for chlamydial infections.

CONCLUSIONS

Betulinic acid can be employed as an antimicrobial agent to *C. trachomatis.* The mode of action of Betulinic acid as an inhibitor of DN topoisomerase IV subunit B (parE), DNA topoisomerase IV subunit A (parC), and Sulfite Reductase (cysJ) will make it an

ideal medication for use in therapeutic applications. Betulinic acid with all its potential benefits addressed can be used as an antimicrobial agent to eradicate the pathogen *C. trachomatis* which are recalcitrant to treatment.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Riau for providing the facilities and support needed to complete this research.

CONFLICT OF INTEREST

The authors state that they have no personal interests that could have influenced their research.

REFERENCES

Adepoju, F.O., Duru, K.C., Li, E., Kovaleva, E.G., Tsurkan, M. V., 2023. Pharmacological Potential of Betulin as a Multitarget Compound. Biomolecules 13(7), 1-30. https://www.mdpi.com/2218-273X/13/7/1105

Benamri, I., Azzouzi, M., Sanak, K., Moussa, A., Radouani, F., 2021. An Overview of Genes and Mutations Associated with Chlamydiae Species' Resistance to Antibiotics. Annals of Clinical Microbiology and Antimicrobiology 20(1), 1-12. https://pubmed.ncbi.nlm.nih.gov/34479551/

Bisacchi, G.S., Manchester, J.I., 2015. A New-Class Antibacterial Almost. Lessons in Drug Discovery and Development: A Critical Analysis of More than 50 Years of Effort toward ATPase Inhibitors of DNA Gyrase and Topoisomerase IV. ACS Infectious Disease 1(1), 4–41. https://pubmed.ncbi.nlm.nih.gov/27620144/

D'Atanasio, N., Joannon, A.C.D., Sante, L.D., Mangano, G., Ombrato, R., Vitiello, M., Bartella, C., Magarò, G., Prati, F., Milanese, C., Vignaroli, C., Di Giorgio, F.P., Tongiani, S., 2020. Antibacterial Activity of Novel Dual Bacterial DNA Type II Topoisomerase Inhibitors. PLoS One 15(2),1-9. https://pubmed.ncbi.nlm.nih.gov/32074119/

Devi, S.R., Priyadharsini, V.J., 2022. Virtual Screening to Identify Protein Targets in Aggregatibacter Actinomycetemcomitans Interacting with Berberine. Journal of Pharmaceutical Negative Results 13(7), 2159-2166. https://www.pnrjournal.com/index.php/home/article/view/4898

Grossman, S., Fishwick, C.W.G., McPhillie, M.J., 2023. Developments in Non-Intercalating Bacterial Topoisomerase Inhibitors: Allosteric and ATPase Inhibitors of DNA Gyrase and Topoisomerase IV. Pharmaceuticals 16(2), 1-17. https://pmc.ncbi.nlm.nih.gov/articles/PMC9964621/pdf/pharmaceuticals-16-00261.pdf

Grygiel-Górniak, B., Folga, B.A., 2023. *Chlamydia trachomatis* An Emerging Old Entity? Microorganisms 11(5), 1-12. https://pubmed.ncbi.nlm.nih.gov/37317257/

Jespersen, M.C., Peters, B., Nielsen, M., Marcatili, P., 2017. BepiPred-2.0: Improving Sequence-Based B-Cell Epitope Prediction Using Conformational Epitopes. Nucleic Acids Research 45(1), 24–29. https://pubmed.ncbi.nlm.nih.gov/28472356/

Jury, B., Fleming, C., Huston, W.M., Luu, L.D.W., 2023. Molecular Pathogenesis of

Chlamydia trachomatis. Frontiers Cellular and Infection Microbiology 13, 1-12. https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2023.1281823/full

Kokot, M., Anderluh, M., Hrast, M., Minovski, N., 2022. The Structural Features of Novel Bacterial Topoisomerase Inhibitors That Define Their Activity on Topoisomerase IV. Journal of Medical Chemistry 65(9), 6431–6440. https://pubmed.ncbi.nlm.nih.gov/35503563/

Lou, H., Li, H., Zhang, S., Lu, H., Chen, Q., 2021. A Review on Preparation of Betulinic Acid and Its Biological Activities. Molecules 26(18), 1-17. https://www.mdpi.com/1420-3049/26/18/5583

Oliveira-Costa, J.F., Meira, C.S., Neves, M.V.G., Reis, B.P.Z.C., Soares, M.B.P., 2022. Anti-Inflammatory Activities of Betulinic Acid: A Review. Frontiers in Pharmacology 23(13), 1-12. https://pubmed.ncbi.nlm.nih.gov/35677426/

Orillard, E., Tan, M., 2016. Functional Analysis of Three Topoisomerases That Regulate DNA Supercoiling Levels in Chlamydia. Molecular Microbiology 99(3), 484–496. https://pubmed.ncbi.nlm.nih.gov/26447825/

Praja, R.K., Rosalina, R., 2022. Bioinformatics Investigation of Virulence Factors of Propionibacterium Acnes Targeted by Epigallocatechin-3 Gallate (EGCG). [Proceeding]. 1st Polkesraya International Conference, Palangkaraya.

Rodrigues, G.C.S., Maia, M., Souza, T.A., Lima, E., Santos, L.E.C.G., Silva, S.L., Silva, M.S., Filho, J.M.B., Junior, V., Scotti, L., Scotti, M.T., 2023. Antimicrobial Potential of Betulinic Acid and Investigation of the Mechanism of Action against Nuclear and Metabolic Enzymes with Molecular Modeling. Pathogens 12(3), 1-21. https://www.mdpi.com/2076-0817/12/3/449

Rodrigues, R., Marques, L., Vieira-Baptista, P., Sousa, C., Vale, N., 2022. Therapeutic Options for *Chlamydia trachomatis* Infection: Present and Future. Antibiotics 11(11), 1-16. https://www.mdpi.com/2079-6382/11/11/1634

Szklarczyk, D., Santos, A., Mering, C., Jensen, L.J., Bork, P., Kuhn, M., 2016. STITCH 5: Augmenting Protein–Chemical Interaction Networks with Tissue and Affinity Data. Nucleic Acids Research 44(D1), D380–D384. https://pubmed.ncbi.nlm.nih.gov/26590256/

Witkin, S.S., Minis, E., Athanasiou, A., Leizer, J., Linhares, I.M., 2017. *Chlamydia trachomatis*: The Persistent Pathogen. Clinical and Vaccine Immunology 24(10), 1-17. https://pubmed.ncbi.nlm.nih.gov/28835360/

[WHO] World Health Organization., 2025. Chlamydia [WWW Document]. www.who.int. [Diakses Agustus 2025].

Xia, X., 2017. Bioinformatics and Drug Discovery. Current Topics in Medical Chemistry 17(15), 1709–1726. https://pubmed.ncbi.nlm.nih.gov/27848897/