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Targeting *Gardnerella Vaginalis* with Jatamansin for the Treatment of Bacterial Vaginosis

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Abstract

One of the most prevalent infections in the vagina is bacterial vaginosis which causes immense physical and psychosocial discomfort in reproductive women and raises the possibility of preterm birth, pelvic inflammatory disease, and other conditions. Many studies show that among the bacterial species that cause this disease, Gardnerella vaginalis is the major cause of bacterial vaginosis. This study aims to identify potential protein targets for treating bacterial vaginosis disease using phytochemical compounds. The potential protein target of G. vaginalis is identified through several analyses using bioinformatics tools. It was interacted against the existing 74 phytochemical compounds which were already used in studies related to vaginal infection, G. vaginalis, bacterial vaginosis by using molecular docking approach and stable interactions were analyzed using MD simulation. The potential protein target is 30s ribosomal s15, and the top 5 potential phytochemical compounds are Luteolin, Paulowin, Jatamansin, Apigenin, and Kaempferol were identified, and their interactions were visualized. Molecular Dynamics shows that among the 5 compounds, Jatamansin is stable and interacts well with the target. Jatamansin is a potential phytochemical that interacts well and has good pharmacokinetic properties against the 30s ribosomal s15 G. vaginalis protein to inhibit bacterial vaginosis infection.

Keywords: *Bacterial Vaginosis, Gardnerella Vaginalis, Phytochemicals, 30s Ribosomal s15.*

Introduction

A vaginal dysbiosis caused by high concentrations of facultative and strict anaerobic bacteria (such as *Gardnerella vaginalis*, *Prevotella* spp., *Atopobium vaginae*, and others) replaces healthy, lactic acid- and hydrogen peroxide-producing *Lactobacillus* spp. in cases of bacterial vaginosis (BV), the most common cause of vaginal discharge. It is still unclear exactly what causes BV, even after over 60 years of research [1]. This makes bacterial vaginosis (BV) one of the most common and frequent vaginal infections, afflicting approximately 30% of women worldwide [2].

Throughout their lives, between 23-29% of women of reproductive age worldwide experience BV. Nonetheless, between 50 and 60 percent of adult women experience a UTI at least once in their lives [3]. Clinicians and researchers concur that more potent treatments for BV are desperately needed to enhance cure rates and, eventually, lessen harmful health effects [2].

The prevalence differs geographically: Europe and Central Asia, between 23%-29%, East Asia and the Pacific, 24%, the Middle East and North Africa, 25%, North America, 27%, and South Asia, 29%. In North America, the series is 33% Black, 31% Hispanic, 23% White,

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and 11% Asian [4]. In light of the aforesaid fact, it becomes very necessary to raise awareness about these practices as, according to various studies, they may be associated with low socio-demographic conditions, poor menstrual health management, and older age [5].

Topical treatment options include metronidazole gel 0.75% once daily for 5 days or clindamycin cream 2% at bedtime for 7 days. 500 mg of oral metronidazole given twice a day for seven days is an additional treatment option. The recommendation to avoid alcohol while receiving treatment has changed [7].

The most typical reason for abnormal vaginal discharge is BV. In addition, vaginal itching, elevated pH, and malodor are characteristics of symptomatic BV [7]. Risk factors are linked to various forms of vaginal dysbiosis or infections, such as vulvovaginal candidiasis (VVC), herpes simplex virus (HSV), aerobic vaginitis (AV), and BV [8]. It may raise the risk of chorioamnionitis, preterm labor, mid-trimester miscarriages, post-abortion pelvic inflammatory disease, and vaginal cuff infection following a hysterectomy [9].

The species was first isolated by Leopold in 1953 from male urine and female cervix swabs, and later termed *Haemophilus vaginalis* by Gardner and Dukes in 1955, who showed its association with BV. It was first placed in the genus *Corynebacterium* but later renamed *G. vaginalis* and placed in the new genus *Gardnerella*. It is rod-shaped, Gram-variable, and the cell wall contains a thin layer of peptidoglycan with a high GC content. This organism contains a variety of virulence factors; of these, sialidase and vaginolysin have been most intensively investigated [10].

A review article elaborates on the association of *Gardnerella* with BV and also discusses the genotypic diversity of this organism along with the limitation of sequence-based studies. It has been proposed that in some cases; the symptoms of vaginal shedding are also mimicked by *Gardnerella vaginalis* [11]. The growth of *G. vaginalis* in the vagina is aided by biofilm formation. High levels of cytotoxicity and increased adherence to epithelial cells indicate that *G. vaginalis* is more virulent than other anaerobes [12].

Proteins found in the cytoplasm are essential for preserving cellular metabolism and functionality. They participate in several metabolic processes that are essential to the survival and expansion of bacteria. By focusing on these proteins, it is possible to interfere with vital functions and cause bacterial death or stasis. Generally speaking, cytoplasmic proteins are better to interact with when designing drugs than membrane proteins, which frequently require intricate interactions with lipid bilayers. This accessibility makes it easier to generate peptides or tiny compounds that can effectively block their action [13, 14].

The multiunit structure ribosome converts mRNA into protein, which is crucial for cell division, proliferation, apoptosis, development, and transformation [15]. A few ribosomal proteins that are involved in protein translation and assembly have also been demonstrated to have other functions, such as blocking tumour cells, viruses, bacteria, fungi, and parasites. As such, they could be categorized as antimicrobial peptides (AMPs). Nevertheless, little is known about the exact mechanism of action of ribosomal proteins as AMPs [16].

This study suggests that phytochemicals can effectively bind to and inhibit key virulence proteins of *G. vaginalis*, the primary pathogen of BV. It aims to determine if molecular docking and simulation can identify phytochemicals with strong binding affinity, potentially leading to new BV treatments. Using computational techniques, this research will focus on promising phytochemical candidates, differing from previous studies that targeted ribosomal proteins.

Methodology

This study identified target proteins and phytochemicals from research journals using

literature mining techniques that extracted and analyzed relevant data to understand their interactions as documented in earlier studies.

Protein Target Identification

A study by Marín E. et al. isolated 261 *G. vaginalis* proteins by a cell shaving approach and predicted their localization with PSORT and Gpos-mPloc. Here, this protein data was used to further analyze virulence factors and non-orthologs that finally identify one protein as a potential target to treat bacterial vaginosis.

The FASTA sequence of the 30S ribosomal protein S15 (BAQ32844) was retrieved from NCBI and searched against the PDB database using HHpred and modelled the 3D structure using Modeller [17, 18]. Using the Ramachandran plot produced by PDBsum, the quality of the modelled structure was evaluated [19]. And refined using the Modrefiner tool [20]. EcoCyc is an online bioinformatics database that includes detailed information on the genomes and biochemical pathways of a great number of species, including *Gardnerella vaginali*s. This resource has provided detailed data on genes, gene products, metabolites, reactions, operons, metabolic pathways, gene regulation, gene essentiality, and nutrient conditions [21]. The application of EcoCyc to studies on *Gardnerella vaginalis* 30S ribosomal protein S15 targeting will inform one about molecular machinery, regulators, and metabolic pathways in this organism; this enhances understanding and effective identification of therapeutic targets.

The model of the target protein's 3D structure was uploaded to the web server of P2Rank for predicting the ligand binding site [22]. P2Rank is a machine learning-based, template-free approach that clusters protein surface points with respect to their likelihood of being bound together and reveals possible binding sites [23]. The highest predicted binding pocket was selected for further investigation and visualized using Chimera, a molecular visualization software [24].

Ligand Identification

Articles from Databases such as PubMed and Google Scholar were searched using keywords related to *Gardnerella vaginalis*, bacterial vaginosis, and phytochemicals. From the relevant research articles and Dr. Duke's Phytochemical and Ethnobotanical Database, a list of 74 phytochemical compounds and their corresponding plant sources was compiled. The phytochemical properties, ADMET (absorption, distribution, metabolism, excretion, and toxicity) data, were obtained by Swiss ADME and Protox web servers. 3D structures of all the phytochemicals collected from the Pubchem database [25-30]

Docking and Simulation

The 30S ribosomal protein S15 structure was prepared for docking using Biovia, and docking was performed using PyRx, where the phytocompounds bound at the predicted site [31, 32]. Compounds meeting the Lipinski rule of 5 and with a lower toxicity class are considered potential candidates. Binding interactions were visualised using Biovia. The top 5 Phytochemical compounds (Luteolin, Paulowin, Jatamansin, Apigenin, and Kaempferol) underwent 100 ns molecular docking simulations using GROMACS (version 2024.2). The RMSD, RMSF, RoG, and Hydrogen bond plots identified one compound as potentially stable.

Results

Target and Binding Site Identification

The 30S ribosomal protein S15 (BAQ32844.1) from *Gardnerella vaginalis* is crucial for protein synthesis, consisting of 89 amino acids with essential binding sites for rRNA and other ribosomal proteins. Targeting this protein may help identify phytochemicals that disrupt ribosome function, serving as potential antimicrobial agents against bacterial vaginosis. Encoded by the rpsO gene, S15 can bind to its mRNA and inhibit the translation of

its mRNA, creating a feedback loop that maintains protein levels. Knocking out the rpsO gene leads to growth failure in nutrient-rich environments, highlighting the protein's vital role in bacterial survival. Thus, inhibiting S15 could impede the protein synthesis machinery of *Gardnerella vaginalis*. The template 8B0X_O (Translating 70S ribosome chain "O" of Escherichia coli) was selected and used to model the target. The 3D structure was validated by the Ramachandran plot showed 93% of residues in the favoured region, indicating a good protein structure. The P2Rank's Pocket 1 consisted of 10 key residues (Table 1) that were identified as the primary binding site for potential ligands.

Table 1. 10 Key Residues

Residue_label	1	39	42	43	45	46	49	52	55	59
Residue_name	MET	LEU	HIS	LEU	GLU	HIS	ASP	SER	GLY	MET

Ligand Properties

Accordingly, the 74 phytochemicals have been prioritized based on their known antimicrobial activity against *Gardnerella vaginalis* or the ability of their ingredients to treat bacterial vaginosis associated with vaginal infection. Favourable ADMET properties, including bioavailability, safety, Lipinski's violation (drug-likeness), and toxicity endpoints. All the compounds were docked against the S15 protein at the binding site residues mentioned in Table 1.

Computational Analysis

The screening was based on the phytochemicals' dock score in kcal/mol, class of toxicity, and violation by Lipinski. The top 5 (high dock score) compounds (Table 2) were taken for further simulation process to understand the stable interactions between the S15 protein and the phytochemicals.

Table 2. The Top 5 (High Dock Score) Compounds

Phytochemical Compounds	**Docking Score**	**Lipinski**	**Toxicity Class**
Luteolin	-6.9	**Violation passed**	5
Paulowin	-6.9		3
Jatamansin	-6.8		4
Apigenin	-6.7		5
Kaempferol	-6.5		5

The RMSD profile (Figure 1) shows that Jatamansin maintains a lower RMSD throughout the simulation, suggesting a more stable binding pose to the target protein compared to other compounds. RoG plot (Figure 2) indicates that Jatamansin consistently exhibits a lower RoG value than Luteolin, Apigenin, Paulowin, and Kaempferol, indicating a more compact binding conformation with the 30S ribosomal protein S15. Jatamansin was visualized with Biovia, and before the simulation, it formed hydrogen bonds with ARG63, carbon-hydrogen bonds with SER33, pi-alkyl interactions with ALA37, and van der Waals interactions with ILE11, TYR15, ALA30, LYS34, LEU85, and LEU87 (Figure 3). During and after the simulation, no hydrogen bonds were observed, which suggests that the complex adapts to a different stable conformation in the dynamic environment. The RMSF chart (Figure 4) shows that Jatamansin has less flexibility compared to the other compounds, suggesting stable and constrained interactions with the target. This stability makes Jatamansin a promising candidate for further investigation and development.

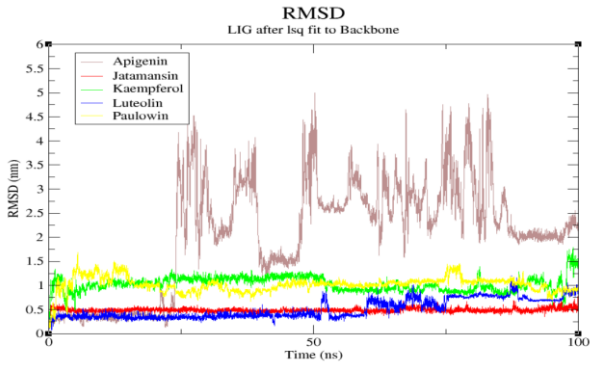

Figure 1. Plot of the Root Mean Square Deviation (RMSD) of Five Phytochemical Compounds Binding to 30S Ribosomal Protein S15

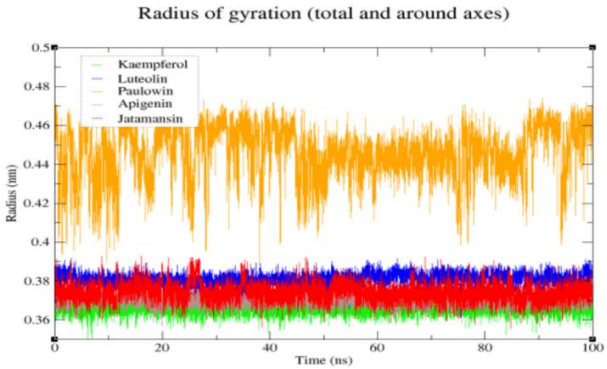

Figure 2. Radius of Gyration (RoG) Plot of 5 Phytochemical Compounds Binding to 30S Ribosomal Protein S15

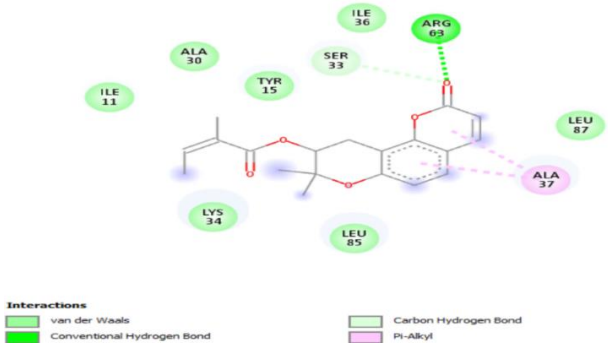

Figure 3. Interaction of Jatamansin Compound against 30S Ribosomal Protein S15 before Simulation

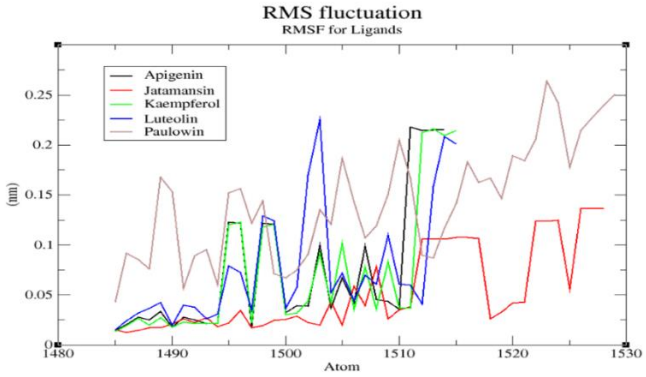

Figure 4. RMSF Plot of Five Phytochemical Compounds Bound to 30S Ribosomal Protein S15

Discussion

The current study aimed at identifying phytochemical compounds as potential novel treatments against bacterial vaginosis caused by *Gardnerella vaginalis*. 30S ribosomal protein S15 was identified as a promising drug target since it can bind to its mRNA and inhibit the translation of its own mRNA. In particular, some phytochemicals such as Luteolin, Paulowin, Jatamansin, Apigenin, and Kaempferol showed good binding with the protein, proving the possibility of their inhibitor activity. Simulation results revealed that Jatamansin folded into a compact conformation with stable binding without the formation of hydrogen bonds, thus proving another mode of binding.

Nardostachys jatamansi, commonly known as Indian spikenard, is a perennial herb from the Valerianaceae family, growing 10–60 cm tall. It grows in the alpine Himalayas—from Punjab to Sikkim and Bhutan, between altitudes of 3000–5000 meters above mean sea level. The various properties it is reported to possess include the following: antidepressant, anticonvulsant, anti-Parkinson's, hepato-protective, antibacterial, and cardio-protective effects [33]. Further in vitro and in vivo studies are hence needed to unravel the binding mechanism of Jatamansin to its inhibitory action on the protein S15 of *G. vaginalis*.

Geethashree S. et al. (2023) investigated the microbiome of bacterial vaginosis using MetaPhlAN2 for taxonomic classification and HUMAnN2 for pathway analysis and retrieved microbial abundance and diversity information. Shotgun metagenomic sequencing was used to identify bacterial diversity, and homology modelling of BV gene receptors like LAP3 and PEPD identified putative Ayurvedic herb ligands. These molecular docking studies revealed potential phytochemicals like kaempferol, oxalic acid, angelic acid, orselol, and chlorozotocin. These findings advance our understanding of the BV microbiome and provide drug-development targets for the treatment of this common infection [34].

In a gel-free proteomic study, 261 surface-associated proteins in *G. vaginalis* were identified through direct trypsin digestion, while motifs characteristic of surface-exposed proteins were found. Bioinformatics analysis puts forth potential diagnostic markers and therapeutic targets for bacterial vaginosis. This approach provided insight into the pathogenesis of the disease and proposed promising treatment strategies [35].

Shvartsman et al. (2023) investigated the *Gardnerella* genus concerning genetic and phenotypic variability, differences in virulence factors related to bacterial vaginosis. Apart from the difference in vaginolysin production and its effects on mucosal immunity, this work identifies immune responses involving cytokine induction and cervical remodelling. These insights provided valuable context for identifying protein targets in BV treatment research.

Conclusion

In this study, the 30S ribosomal protein S15 of *Gardnerella vaginalis* has been identified as a target candidate for the management of bacterial vaginosis. Molecular docking and simulation studies showed jatamansin as a strong binder. The research integrates bioinformatics and molecular simulations for the identification of jatamansin as a promising therapeutic agent and,

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Conflict of Interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus;

membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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