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Therapeutic Adjuvant Potential and Herb-Drug Interaction of *Withania somnifera* (L.) Dunal (*Ashwagandha*) co-administered with First-Line Anti-Tubercular Drugs – an *In silico* Exploration

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Abstract

Tuberculosis (TB) remains a major global health burden despite the availability of effective drugs and policy interventions. Prolonged treatment duration, the emergence of drug resistance, and adverse effects of anti-tubercular drugs (ATDs) highlight the need for supportive adjuvants. Objective: We performed an *in-silico*investigation of *Withania somnifera* (Ashwagandha), a popular rejuvenator herb (*Rasayana*) in Ayurveda with established antioxidant, anti-inflammatory, and immunomodulatory properties for its potential role in TB management. Molecular docking was performed to evaluate interactions of key Ashwagandha phytoconstituents with human drug-metabolizing enzymes and transporters influencing the pharmacokinetics of first-line TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol), as well as with *Mycobacterium tuberculosis* proteins essential for bacterial survival and virulence. Several compounds, including 12-Deoxy Withastramonolide, Withanoside IV, Withanolide A, and Withaferin A, exhibited favorable binding to both sets of targets, suggesting possible antimycobacterial activity and beneficial herb–drug interactions. These findings indicate that Ashwagandha may provide dual benefits direct inhibition of the pathogen and enhanced ATD availability supporting its potential as an adjuvant in TB therapy warranting clinical evaluation.

Introduction

Pulmonary tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the most widespread infectious diseases worldwide. The World Health Organization (WHO) Tuberculosis Report 2024 highlights that TB continues to pose a major global health challenge. In 2022, approximately 10.7 million new cases were recorded, rising to about 10.8 million in 2023, highlighting the persistent global health burden of the disease India remains the highest-burden country, accounting for 26% of global cases with an estimated 2.7 million incidents and 3.2 lakh deaths in 2023 under the National Tuberculosis Elimination Programme (NTEP). The disease is frequently associated with comorbid conditions such as diabetes, HIV/AIDS, and malnutrition, all of which worsen disease progression and complicate treatment outcomes. In response to this challenge, India has intensified TB control efforts through multiple interventions under NTEP, including early case detection, molecular diagnostics such as CBNAAT (GeneXpert), universal drug susceptibility testing, treatment adherence monitoring, and nutritional support through *Nikshay Poshan Yojana*. Despite the availability of effective drugs and policy interventions, TB remains a major global health burden and a leading cause of mortality, especially in low- and middle-income countries.[1–3]

WHO recommends a 6-month regimen for drug-susceptible TB, consisting of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). For drug-resistant TB, WHO advises longer regimens with second-line drugs based on drug susceptibility testing. Long-term use is linked to various side effects such as hepatotoxicity, gastrointestinal disturbances, peripheral neuropathy, arthralgia, hypersensitivity reactions, and visual disturbances.[4] These adverse effects may compromise treatment outcomes. These conventional anti-tubercular drugs primarily target the pathogen and may not adequately address the systemic consequences of disease, including chronic inflammation, oxidative stress, cachexia, neurocognitive decline, and immune dysregulation. Extended use of first-line ATD regimens has contributed to the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), limiting treatment options and increasing mortality.[5]

In response to these limitations, global research focused on novel therapeutic strategies, including new drug regimens, vaccines, and host-directed therapies (HDTs) aimed at improving treatment outcomes and shortening therapy duration. Several clinical trials are currently investigating innovative approaches, including new vaccine candidates such as the M72/AS01E tuberculosis vaccine and host-directed adjunct therapies like metformin and vitamin D, which aim to modulate host immune responses and enhance bacterial clearance.[6]

Alongside these approaches plant-derived bioactive compounds and traditional medicinal systems are also increasingly being explored as potential adjunct therapies. Many herbal compounds exhibit immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial properties, which may complement conventional anti-tubercular therapy and improve treatment tolerance.[7] Several natural and synthetic compounds have been investigated as adjuncts to standard TB therapy, demonstrating immune-supportive and anti-inflammatory effects in both in vitro and in vivo studies.[8, 9] Most of these approaches target individual pathways and do not comprehensively address the complex pathophysiological features of TB, including immune dysregulation, oxidative stress, treatment-related toxicity, and overall host health. These limitations highlight the need to explore multitarget therapeutic agents capable of addressing multiple disease hallmarks simultaneously and host health.

Withania somnifera (WS) (Ashwagandha), a popular rejuvenator herb (*Rasayana*) with established antioxidant, anti-inflammatory, and immunomodulatory properties offer holistic approach to managing TB hallmarks, including immune dysregulation, chronic inflammation, oxidative stress, and treatment-related systemic effects.[10] By targeting several disease hallmarks simultaneously, WS may support host resilience during TB therapy and potentially enhance the efficacy and tolerability of standard antitubercular treatment. There are few studies have explored WS as an adjuvant to first-line TB therapy which highlight its potential as a strategic addition to first line therapy[11, 12]. However, these studies do not explore underlying molecular mechanisms and potential HDI. Understanding these aspects is essential for ensuring the safety and effectiveness of such integrative therapeutic approaches

The present study aimed to investigate the potential role WS as a therapeutic adjuvant in TB management using an *in silico* molecular docking approach. Key phytoconstituents of WS were evaluated for their binding affinity against essential *Mycobacterium tuberculosis* proteins involved in bacterial survival and virulence. In addition, potential herb-drug interactions were explored by assessing the interactions of these phytoconstituents with human pharmacokinetic targets, including major drug transporters and drug-metabolizing enzymes involved in the absorption, distribution, metabolism, and excretion (ADME) of first-line antitubercular drugs. By integrating pathogen-target and pharmacokinetic-target docking analyses, this study provides mechanistic insights into the antimycobacterial potential of WS phytoconstituents. It also evaluates their possible influence on the pharmacokinetics (PK) of standard antitubercular therapy.

1. Method

1.1. Study Design and Docking Workflow

The study design and docking workflow were employed to assess the interaction of WS phytoconstituents with (a) *Mycobacterium tuberculosis* targets and (b) human PK (ADME) related targets. The study workflow is outlined below, followed by detailed docking methodology for each set of targets.

Molecular docking of phytoconstituents of Ashwagandha was performed with the human drug metabolizing enzymes (CYPs) and transporters which influence the PK of first line TB drugs. The selected docking proteins are P-glycoprotein (P-gp), Organic Anion Transporting Polypeptide 1B1 (OATP1B1), and cytochrome P450 enzymes (CYP2C9, CYP2C19, and CYP3A4). Rifampicin (RIF) is known to strongly induce several drug-metabolizing enzymes, particularly cytochrome P450 isoforms CYP3A4 and CYP2C9 in the liver and small intestine. It enhances the expression of certain drug transporters, including P-glycoprotein in both intestinal and hepatic tissues.[13] INH undergoes primary metabolism via N-acetyltransferase 2 (NAT2) however it can inhibit CYP2C19 and CYP2C9, altering the metabolism of co-administered drugs.[14] PZA and EMB are less extensively metabolized but may interact with hepatic or intestinal transporters. WHO recommends the 6 months regimen of ATD for drug susceptible TB. Prolonged administration of these drugs may influence the activity of drug metabolizing enzymes and various drug transporters. Therefore, WS phytochemicals were evaluated for their potential interaction with key drug metabolizing enzymes and drug transporters associated with ATD disposition. Molecular docking against these targets provides a mechanistic rationale for assessing the safety and compatibility of co-administration with WS.

1.1.1. Selection and Preparation of Target Proteins

A total of nine target proteins were selected for this study to evaluate both HDI potential and antitubercular activity. The proteins included human transporters and metabolic enzymes involved in drug ADME processes include P-gp (PDB ID: 6C0V), OATP1B1 (PDB ID: 8K6L), Cytochrome P450 enzymes CYP2C9 (PDB ID: 5W0C), CYP2C19 (PDB ID: 4GQS), and CYP3A4 (PDB ID: 7UFC) as well as Mtb targets such as Arabinosyl Transferase C (AraC) (PDB ID: 3PTY), Protein Kinase A (PknA) (PDB ID: 4OW8), Mycobacterial Glutamine Synthetase (GlnA) (PDB ID: 3ZXR), and Proteasomal ATPase (PDB ID: 5KWA). All protein structures were retrieved in PDB format from the RCSB Protein Data Bank and selected based on resolution and biological relevance.

Protein preparation was conducted using AutoDock Tools (version 1.5.7). This involved the removal of water molecules, addition of polar hydrogen atoms, and assignment of Kollman charges. After cleaning and optimization, each protein structure was saved in PDBQT format for docking. The grid box for each protein was positioned to encompass the entire protein structure, as blank docking was performed to allow exploration of all potential binding sites.

1.1.2. Ligand Preparation

The major bioactive phytoconstituents of WS were identified through literature and obtained from the PubChem database in SDF format. These structures were converted into PDB format and subjected to energy minimization using Chimera software (version 1.15) employing the AMBER force field. Subsequently, ligand preparation was performed using AutoDock Tools (version 1.5.7), which included the addition of Gasteiger charges, torsion setting, and conversion into PDBQT format.

1.1.3. Docking programme

Docking program were performed using AutoDock Vina. Each ligand was docked to the prepared protein targets, and binding affinities were recorded in kcal/mol. The docking protocol ensured consistent grid spacing, exhaustiveness, and receptor flexibility settings across all simulations. Docking scores and binding conformations were saved for further analysis.[15]

1.1.4. Visualization of Protein-Ligand Interactions

The resulting protein-ligand docked complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer (2021). This enabled the identification of key molecular interactions such as hydrogen bonds, hydrophobic interactions, and pi-stacking, providing insights into the potential binding modes and strength of interaction between WS compounds and the selected protein targets.

1.2. Clinical Correlation of TB Treatment Algorithms and PK–ADME Profiles

Alongside the *in silico* analysis, we reviewed standard tuberculosis treatment algorithms and the pharmacokinetic (PK; A, D, M, E) profiles of first-line antitubercular drugs (INH, RIF, PZA, and EMB). We studied the reported clinical plasma levels, half-lives, therapeutic range from published literature and interpreted them in the context of current treatment regimens. We further explored how WS might influence these PK; A, D, M, E parameters, cross-checking *in silico* observations on the level of preclinical and clinical correlation. This approach allowed us to clinically interpret potential WS-drug interactions, as summarized in the Results section (Table 3).

Table 3
Pharmacokinetics of First line ATDs and selected phyto

Pharmacokinetic Parameters	First-line Anti-Tubercular Drugs (Literature data)				*Withania somnifera* (*in silico* data)		
	Rifampicin	**Isoniazid**	**Pyrazinamide**	**Ethambutol**	**12-Deoxy Withastramonolide**	**Withanoside IV**	**Withanolide A**
Clinical dose[38, 39]	10 mg/kg (max 600 mg)	5 mg/kg (max 300 mg)	25 mg/kg (max 2 g)	15 mg/kg (max 1.6 g)	Not reported yet		
Therapeutic plasma range[38, 39]	8–24 µg/mL	3–6 µg/mL	20–50 µg/mL	2–6 µg/mL	Not reported		
Absorption [40] [41] [42] [43] [44] [45]	Small intestine; P-gp, OATPs, OCTs*, MRP	Small intestine (duodenum and proximal jejunum); OATPs, P-gp, MRPs*	Small intestine (duodenum and proximal jejunum), P-gp, OATPs (Limited evidence of transporter involvement as passive diffusion)	Small intestine (duodenum and proximal jejunum); P-gp, OATPs, OCTs*	Small intestine; P-gp substrate Docking Showed good affinity toward P-gp	Small intestine; P-gp substrate, Docking Showed high affinity toward P-gp	Small intestine; P-gp substrate Docking Showed high affinity toward P-gp
					Molecular docking results indicated high binding affinity towards P- antitubercular drugs when co-administered with *Withania somnifera*		
Distribution [40] [41]	Liver, Lungs, placenta, blood-brain barrier; OATPs (1B1, 1B3), P-gp, BCRP*	Liver, lungs, brain; OCT1, OCT2, P-gp, OAT1	Liver, lungs, cerebrospinal fluid (CSF); P-gp, OAT1, OAT3*, OAT4*	Liver, lungs; P-gp, OAT1, OAT3*	Plasma; Not BBB permeant, Docking Showed high affinity toward P-gp	Plasma; Not BBB permeant, Docking Showed high affinity toward P-gp	Plasma; Not BBB permeant, Docking Showed high affinity toward P-gp
					Molecular docking showed good binding affinity towards P-gp, sugg administered with *Withania somnifera*. However, WS is likely fully or		
Metabolism[40] [41] [46]	Liver; CYP3A4, CYP2C19, CYP2B6	Liver; NAT2 (major), CYP3A4, CYP 2C19 (minor)	Liver; Pyrazinamidase (PZase), CYP450 Enzymes	Liver; Cytochrome P450 (CYP) Enzymes: CYP2B6, CYP3A4, and CYP1A2, Aldehyde Dehydrogenase (ALDH)	Liver; Cytochrome P450 Enzymes (CYP450), Glucuronosyltransferases (UGTs) CYP2C9 inhibitor Docking showed high binding affinity toward CYP2C9, CYP2C19, and CYP3A4	Intestinal Tract; Gut Glucosidases Docking showed high binding affinity toward CYP2C9, CYP2C19, and CYP3A4	Liver; Cytochrome P450 Enzymes (CYP450), Glucuronosyltransfer (UGTs) Docking showed high binding affinity towar CYP2C9, CYP2C19, a CYP3A4
					First-line ATDs are metabolized by specific CYP enzymes (Rifampic CYP1A2). *Withania somnifera* phytoconstituents show high affinity f suggest minimal CYP inhibition. [16, 47]		
Excretion [43] [41]	Kidneys, liver (bile), intestines; P-gp, MRP2, OAT1, OAT3*	Kidneys (urine); OAT1, OAT3, P-gp	Kidneys (urine), liver (bile); OAT, OAT3, MRP2, MRP4*, P-gp	Kidneys (urine); OCT2*, P-gp	Renal Excretion (Kidneys), Biliary Excretion (Liver); ABC Transporters - P-gp, MRPs; OATPs	Renal Excretion (Kidneys), Biliary Excretion (Liver); ABC Transporters - P-gp, MRPs; OATPs	Renal Excretion (Kidneys), Biliary Excretion (Liver); ABC Transporters - P-gp, MRPs; OATPs
					First-line ATDs are excreted via kidneys, bile, and intestines involver affinity toward these transporters, suggesting possible competition likely to have already left or started leaving from the body [36, 37] s		

*Not selected for docking due to low resolution structure or unavailability of crystal structure or no human structure available on Protein Data Bank

2. Results

Several selected phytoconstituents, including 12-deoxy withastramonolide, Withanoside IV, Withanolide A, and Withaferin A, exhibited favourable binding to both sets of targets human ADME and *Mtb* proteins (Tables 1 and Table 2). 2D and 3D interaction diagrams for representative compounds are shown in Fig. 1 (human targets) and Fig. 2 (Mtb targets).

Table 1
Docking scores of selected WS phytoconstituents against human ADME targets

Ligands	P-gp (kcal/mol)	OATP1B1 (kcal/mol)	CYP2C9 (kcal/mol)	CYP2C19 (kcal/mol)	CYP3A4 (kcal/mol)
12-deoxywithastramonolide	-9.5	-9.9	-9.6	-9.0	-11.1
27-hydroxywithanone	-8.3	-9.3	-9.3	-8.1	-9.4
Ashwagandhanolide	-11.5	-10.9	-8.9	-10.6	-11.5
Withacoagin	-9.8	-8.8	-10.3	-10.0	-9.1
Withaferin A	-9.3	-9.8	-8.9	-9.4	-8.2
Withanolide A	-9.3	-9.3	-10.0	-9.3	-11.8
Withanolide B	-8.4	-9.4	-9.0	-10.1	-8.9
Withanone	-8.2	-8.8	-8.7	-8.8	-9.3
Withanoside IV	-9.6	-10.9	-9.3	-9.7	-11.7
Withanoside V	-9.6	-10.5	-10.3	-10.3	-8.6

Table 2
Binding score of selected phytoconstituents of WS with targets of Mtb

Ligands	Arabinosyl Transferase C (kcal/mol)	Protein Kinase A (kcal/mol)	Glutamine synthetase (kcal/mol)	Proteasomal ATPase (kcal/mol)
12-deoxywithastramonolide	-10.1	-9.1	-10.9	-9.5
27-hydroxywithanone	-9.7	-8.7	-9.6	-9.0
Ashwagandhanolide	-9.4	-9.9	-11.1	-10.4
Withacoagin	-9.9	-8.7	-10.7	-8.3
Withaferin A	-9.4	-9.4	-8.8	-7.6
Withanolide A	-10.4	-8.5	-10.8	-9.3
Withanolide B	-10.2	-8.6	-9.8	-8.4
Withanone	-9.2	-8.4	-9.7	-8.7
Withanoside IV	-9.7	-8.6	-10.0	-9.7
Withanoside V	-10.4	-10.3	-10.4	-10.3

2.1. Docking with Human ADME-Related Targets

2.1.1. Absorption

Both ATDs and WS phytoconstituents were predicted to be absorbed primarily in the small intestine, with transporters such as P-gp, OATPs, and MRPs implicated in their transport. Among the WS phytoconstituents, Withanolide A, Withanoside IV, Withanone, Withaferin A, and Withanolide B demonstrated high predicted gastrointestinal absorption, whereas 12-Deoxywithastramonolide and Withanoside V exhibited comparatively lower absorption. Molecular docking analysis revealed favourable binding of selected WS phytoconstituents with P-gp (Table 1). Among the evaluated compounds, Ashwagandhanolide exhibited the highest binding affinity toward P-gp. The ligand formed conventional hydrogen bonds with THR55 and SER298, contributing to stable binding within the transporter cavity. In addition, multiple hydrophobic interactions were observed with residues PHE40, PHE193, PHE194, PHE355, ILE190, MET51, VAL345, LEU225, ALA229, ALA348, MET197, and ILE352. These interactions surrounded the steroidal scaffold of Ashwagandhanolide and facilitated its accommodation within the predominantly hydrophobic binding pocket of P-gp, indicating strong ligand transporter interactions.

2.1.2. Distribution

ATDs were widely distributed in tissues including the liver, lungs, brain, and placenta, mediated by P-gp, OATPs, and OCTs. WS phytoconstituents demonstrated limited blood-brain barrier permeability. WS showed high binding affinity for P-gp, indicating possible modulation of transporter activity at sites such as the liver and lungs. During TB infection, P-gp overexpression at granuloma sites may restrict ATD delivery; interaction of WS phytoconstituents with P-gp may potentially influence ATD distribution to these sites.

2.1.3. Metabolism

First-line ATDs undergo metabolism primarily via cytochrome P450 enzymes: RIF by CYP3A4, CYP2C19, and CYP2B6; INH by NAT2 and CYPs; PZA by pyrazinamidase and CYPs; and EMB by CYP2B6, CYP3A4, and CYP1A2. WS phytoconstituents exhibited strong binding affinities toward CYP2C9, CYP2C19, and CYP3A4. Withanolide A and Withanoside IV showed the highest affinities for CYP3A4 (− 11.8 and − 11.7 kcal/mol, respectively), while Withacoagin and

Withanoside V showed strong affinity for CYP2C9. These findings indicate potential modulation of ATD metabolism. Hydrogen bond analysis revealed that CYP3A4 binding was stabilized by interactions with residues Ser119, Arg212, and Thr309, while P-gp interactions involved hydrophobic contacts at Phe336 and Leu975. However, previous *in vitro* studies suggest minimal CYP inhibition by aqueous extract WS, indicating a low risk of clinically significant inhibitory interaction.

2.1.4. Excretion and Pharmacokinetic Comparison

Both ATDs and WS phytoconstituents were excreted through renal and biliary pathways involving P-gp and OATs. Docking predicted high affinity of WS phytoconstituents for these transporters, indicating potential competition during excretion. Importantly, WS phytoconstituents exhibit rapid absorption and short elimination half-lives, whereas ATDs display longer absorption phases and elimination half-lives. Thus, WS is likely to exert hepatoprotective and immunomodulatory effects during the early absorption phase and be largely eliminated before ATDs reach peak plasma levels, minimizing the potential for adverse pharmacokinetic HDI.

2.2. Docking with Mycobacterium tuberculosis Targets

Docking studies demonstrated that WS phytoconstituents exhibit strong multitarget binding toward selected essential Mtb proteins (Table 2). Among these, Withanolide A, Withanoside V, and Ashwagandhanolide consistently showed the most favorable interactions.

2.2.1. Arabinosyltransferase C:

Arabinosyltransferase C is a crucial enzyme involved in the biosynthesis of the mycobacterial cell wall through its role in arabinogalactan formation. All selected phytoconstituents demonstrated favorable binding affinities toward the enzyme, Withanolide A and Withanoside V exhibited the strongest interactions.

Withanolide A formed five conventional hydrogen bonds with residues TYR841, TRP926, ALA922, ASN928, and ARG930, along with two alkyl interactions involving ALA925 and ALA1059. These interactions were primarily mediated through the hydroxyl and carbonyl functional groups present in the molecule, contributing to stable ligand-protein complex formation. Similarly, Withanoside V showed a strong interaction profile, forming eight conventional hydrogen bonds with ARG1010, THR1036, TYR841, SER1047, ASN928, ARG930, and ASP1056. In addition, two alkyl interactions were observed with LEU1012 and PRO1013, along with a van der Waals interaction involving PHE982. The extensive hydrogen-bonding network can be attributed to the multiple hydroxyl groups and ketone functionalities present in the structure of Withanoside V.

The strong binding affinities and extensive interactions observed for Withanolide A and Withanoside V suggest their potential to inhibit Arabinosyltransferase C activity. Inhibition of this enzyme may interfere with arabinogalactan synthesis and consequently disrupt mycobacterial cell-wall formation, highlighting these compounds as promising anti-tubercular candidates.

2.2.2. Protein Kinase A:

Protein Kinase A (PknA) regulates essential processes in *Mycobacterium tuberculosis*, including cell growth, division, and cell-wall synthesis. Among the selected phytoconstituents, Withanoside V exhibited the strongest binding affinity toward PknA. It formed two conventional hydrogen bonds with GLY145 and LYS108, eleven alkyl interactions with ILE19, LEU97, ALA40, VAL98, VAL27, ALA20, and LEU148, and two van der Waals interactions with LYS108 and GLY22. These interactions are likely mediated by the hydroxyl groups, methyl groups, and steroidal nucleus of Withanoside V. The strong binding suggests that Withanoside V may inhibit PknA activity, potentially disrupting cell-wall integrity and bacterial growth.

2.2.3. Glutamine Synthetase:

Glutamine synthetase plays a vital role in nitrogen metabolism and cell wall biosynthesis. Among the phytoconstituents, Ashwagandhanolide exhibited the highest binding affinity for glutamine synthetase. Its interaction was characterized by one conventional hydrogen bond, twelve alkyl, and pi-alkyl interactions, and one pi-sigma interaction. These findings suggest that Ashwagandhanolide could inhibit glutamine synthetase, disrupting nitrogen metabolism and cell wall production.

2.2.4. Proteasomal ATPase:

Proteasomal ATPase is essential for protein degradation and stress management in Mtb. Ashwagandhanolide also showed the strongest binding affinity toward this target, forming two conventional hydrogen bonds and five alkyl and pi-alkyl interactions. This dual-targeted activity of Ashwagandhanolide highlights its potential to combat bacterial stress mechanisms and promote bacterial degradation.

2D and 3D interaction diagrams for representative compounds are shown in Fig. 1 (human targets) and Fig. 2 (Mtb targets), with additional interactions provided in Supplementary Files S1 and S2.

Collectively, these results indicate that WS phytoconstituents may exert dual effects by modulating human ADME pathways relevant to first line ATD pharmacokinetics and by directly targeting multiple essential Mtb proteins, potentially enhancing therapeutic outcomes

2.3. Clinical Correlation

To contextualize the docking findings, the pharmacokinetic parameters of first-line ATDs were compared with the predicted A, D, M, E characteristics of major WS phytoconstituents (Table 3). Standard clinical doses and therapeutic plasma concentration ranges for INH, RIF, PZA, and EMB were used as reference points as displayed in the Table 3

This comparative analysis highlights key phases/ stages where WS may interact with ATDs. During absorption, WS phytoconstituents demonstrated strong binding affinity for P-gp and OATP transporters, which may influence ATD intestinal uptake. In terms of distribution, both ATDs and WS phytoconstituents rely on overlapping transporter systems, particularly in hepatic and pulmonary tissues, raising the possibility of localized competition or synergistic effects. Metabolic interactions may occur via CYP2C9, CYP2C19, and CYP3A4, for which WS phytoconstituents exhibited high affinity; however, previous *in vitro* studies indicate minimal or no inhibitory activity, [16] suggesting a low likelihood of clinically significant metabolic interactions.

WS phytoconstituents were characterized by rapid absorption and a short elimination half-life [22], while ATDs exhibit longer absorption and elimination phases [23]. This temporal difference implies that WS is likely to exert hepatoprotective, immunomodulatory, and transporter-modulating effects during the early phase of ATD absorption, and be largely eliminated before ATDs reach their peak plasma concentrations. This may reduce the likelihood of adverse pharmacokinetic HDI while still offering supportive adjuvant benefits.

3. Discussion

Prolonged treatment duration, the emergence of drug resistance, and adverse effects of ATDs highlight the need for supportive adjuvants like WS. The integration of adjuvant therapies that can modulate host immunity, enhance treatment tolerance, and complement standard chemotherapy represents a promising strategy to improve treatment outcomes. This study provides mechanistic insights into the potential positive HDIs between WS and first-line ATDs, along with *in silico* evidence supporting the direct anti-mycobacterial activity of WS phytoconstituents.

Molecular docking analysis revealed that several WS phytoconstituents exhibit strong binding affinities toward key drug transporters such as P-gp and OATP1B1 as well as cytochrome P450 isoforms CYP2C9, CYP2C19, and CYP3A4. These interactions suggest the potential for WS to influence ATDs pharmacokinetics, particularly by modulating absorption and hepatic metabolism. Clinically, previous studies have reported increased plasma concentrations of INH and PZA, improved sputum conversion rates, and better immunological profiles when WS is co-administered with ATDs, supporting the translational relevance of these findings.(Kumar et al. 2018; Debnath et al.) Clinically, such pharmacokinetic and immunomodulatory effects may translate into faster sputum conversion, reduced hepatotoxicity, and improved patient adherence to prolonged TB regimens. There is limited evidence about the HDI of WS despite its beneficial adjuvant effects. the present study aims to address and clarify this unexplored aspect at the molecular level by a molecular docking study.

Beyond pharmacokinetic modulation, WS phytoconstituents suggest possible antimicrobial activity through interaction with the proteins for the bacterial virulence. These include targets such as AraC, protein kinase A, glutamine synthetase, and proteasomal ATPase, each playing a critical role in bacterial survival, metabolism, and virulence. AraC is a key enzyme in the biosynthesis of the mycobacterial cell wall, catalyzing the formation of arabinogalactan, a polysaccharide that, together with peptidoglycan and mycolic acids, confers structural rigidity and resistance to host defences.[17] Disruption of AraC activity is a well-established strategy to compromise the bacterium's cell wall integrity. Similarly, Protein Kinase A (PknA), a serine/threonine protein kinase, regulates essential cellular processes such as growth, division, and cell wall synthesis, making it indispensable for maintaining bacterial morphology and viability.[18]

In addition to these structural targets, WS phytoconstituents also interact with metabolic and regulatory proteins. GlnA plays a pivotal role in nitrogen metabolism by catalyzing glutamine synthesis and contributes to cell wall biosynthesis through the production of the poly-L-glutamate-glutamine layer. Its inhibition can therefore impact both metabolic and structural pathways. Furthermore, Proteasomal ATPase is essential for degrading damaged proteins and enabling bacterial survival under oxidative and nitrosative stress encountered within the host environment.[19] Targeting this protein could compromise the pathogen's ability to manage cellular stress and maintain virulence. Targeting multiple essential Mtb proteins simultaneously may reduce the occurrence of resistance development and enhance the bactericidal effect of standard ATDs.

This multi-target interaction suggests that WS may exert direct antimycobacterial effects, complementing standard antitubercular therapy, potentially improving treatment efficacy and supporting the reduction of AMR. TB severely impacts multiple body systems (displayed in Fig. 3), while WS offers protective benefits. It reduces general fatigue and cachexia by lowering cortisol[20] and IL-6 [21], boosting ATP[22], and increasing leptin [23]. In the respiratory system, it protects lung tissue and reduces fibrosis by reducing the level of TNF-α, IL-1β, IL-4, IL-13,[21] TGF-β, [24] and increasing the level of IL-10.[25] For the nervous system, it offers neuroprotection and improves cognition by decreasing TNF-α and IL-6,[26] and enhancing BDNF and GABAergic signalling.[26] Cardiovascular issues like pericarditis and pulmonary hypertension are managed through reduced CRP and IL-6 and increased eNOS and NO levels. [27, 28]. It protects the liver by reducing ALT, AST [29] and enhancing antioxidant enzymes (SOD, CAT, GST).[30] In the endocrine system, it supports adrenal function by lowering cortisol[20, 31] and increasing ACTH and DHEA.[32] It strengthens the immune system by enhancing IFN-γ, IL-2, CD4 + T cells, [33] NK cell activity, and regulating IL-10.[34] By mitigating systemic inflammation, protecting lung tissue, and supporting hepatic function, WS may improve overall treatment tolerance, reduce drug-induced complications, and enhance patient resilience during prolonged therapy.

An important consideration in the clinical use of WS alongside ATDs is the temporal dimension of drug interactions. Hepatotoxicity and other adverse effects associated with first-line ATDs generally emerge after several weeks of therapy, once steady-state concentrations are achieved.[35] First-line ATDs exhibit relatively long absorption phases and elimination half-lives.[36] While, WS has a relatively rapid absorption and comparatively short elimination half-life,[37] leading to their faster systemic clearance. Consequently, during the absorption phase of ATDs, WS phytoconstituents may already exert their hepatoprotective, immunomodulatory, anti-inflammatory, and antibacterial effects, supporting host physiology and potentially mitigating early drug-induced hepatic stress. This pharmacokinetic profile suggests that, WS is likely to begin elimination before peak ATD concentrations achieved, thereby minimizing the likelihood of adverse pharmacokinetic herb-drug interactions while still contributing beneficial systemic effects. Moreover, WS has demonstrated favourable safety in clinical studies, with no significant inhibition of major CYP enzymes *in vitro*,[16] supporting its potential compatibility with ATDs when used at appropriate doses and durations.

It is also important to consider the complex interplay between TB pathophysiology and drug disposition. TB-induced changes in gastrointestinal physiology, vascularization of lesions, hepatic metabolism, and renal function can alter drug absorption, distribution, metabolism, and excretion. WS may help counter some of these effects by improving host immune function, reducing systemic inflammation, and supporting organ systems such as the liver and lungs, potentially stabilizing pharmacokinetic variability during treatment.

4. Limitations of studies

A major limitation is *in silico* analysis considers isolated phytoconstituents (like Withanolides, Withanosides, etc), whereas herbal extracts (like aqueous extract of WS) contain a complex mixture of numerous phytoconstituents. Molecular docking considers pure phytomolecules interacting with target proteins. Herbal extracts contain a complex mixture of multiple constituents, with the concentration of active phytomolecules often being very low. The therapeutic effects of herbal formulations are influenced not only by the major active compounds but may also by the interactions among various minor constituents. Thus, the observed binding affinities observed in *in silico* may not directly translate into biological efficacy when using whole extracts, and the actual pharmacokinetic behaviour could be different due to factors like poor bioavailability, metabolism, and interaction with other extract components. Therefore, these findings need to be interpreted cautiously, and further experimental validation is essential.

Another key limitation of this study is that while the *in silico* approach predicts herb-drug interactions of WS with standard first-line ATDs, actual clinical regimens are highly individualized, often involving variations such as INH or RIF resistance, modified treatment durations, or extended therapies in conditions like HIV, malnutrition, diabetes, or hypertension. These factors significantly influence body physiology, immune responses, and drug pharmacokinetics: however, current *in silico* models do not account for such complexities. Additionally, clinical practice employs molecular diagnostics (e.g., MTBDR, MTBDRsl) to detect resistance-related mutations (such as those in *katG*) and considers pharmacogenomic factors like acetylator status which affects INH metabolism, and is not simulated by *in silico* methods. As a result, while these models provide valuable insights into predictive interactions, they do not capture the personalized pharmacokinetic–pharmacodynamic variability of real-world therapeutic settings, underscoring the need for validation in clinically relevant scenarios.

Therefore, while these findings provide valuable predictive insights, experimental validation including *in vitro* enzyme/transporter assays, *in vivo* pharmacokinetic studies, and clinical trials is essential to confirm the nature and magnitude of these interactions. The investigators are working on a detailed pharmacokinetics study (CTRI/2024/01/061852) on WS that may add newer insights into phytoconstituent behaviour. There is a need for a robust clinical trial for better understanding of WS as an adjuvant in TB management. This study will bridge the knowledge gap and may be a milestone in long awaited TB eradication.

5. Conclusion

This *in silico* study provides insights into the pharmacokinetic interactions between *WS* and first-line ATDs. The findings suggest positive HDIs are mediated by shared transporter pathways. Co-administration of WS with first-line antitubercular drugs may result in positive HDI due to its effects on key drug transporters and metabolizing enzymes. Molecular docking studies show that phytoconstituents of WS exhibit higher affinity for P-gp and OATP1B1, potentially reducing drug efflux, increasing drug absorption, and interfering with hepatic drug uptake and metabolism. Also, these phytoconstituents showed strong interactions with CYP2C9, CYP2C19 and CYP3A4 which might decrease the metabolism of antitubercular drugs. These interactions could alter the pharmacokinetics of the drugs, increasing their bioavailability and plasma concentration, which may impact therapeutic efficacy by reducing the dose and related adverse events of ATD.

The molecular docking results revealed that the discussed phytoconstituents of WS exhibit a high binding affinity towards Arabinosyltransferase C, protein kinase A, glutamine synthetase, and proteasomal ATPase suggesting that WS may interact or inhibit these enzymes which are responsible for the survival and virulence of Mtb. These findings indicate that WS may possess antitubercular properties. Thus, the WS as a TB adjuvant improves safety as well as efficacy of conventional ATD.

in vivo studies are necessary to confirm these findings and evaluate their clinical significance. Because *in silico* studies typically evaluate interactions based on individual phytomolecules. However, in the case of aqueous extracts, such interactions are minimal due to the low concentration of phytomolecules in the extract. The extent of interactions depends on the type and enrichment of the extraction process. Therefore, the potential for HDI between the aqueous extract of WS and first-line ATDs may very low suggesting the potential of WS as an adjuvant therapy. The findings suggest clinical evaluation of WS aqueous extract as ATD adjuvant in a randomized controlled clinical trial following a robust protocol.

Abbreviations

ADME - Absorption, Distribution, Metabolism, and Excretion

AraC - Arabinosyl Transferase C

ATDs - Anti-Tubercular Drugs

BCRP - Breast Cancer Resistance Protein

CBNAAT - Cartridge-Based Nucleic Acid Amplification Test

CNS - Central Nervous System

CYP - Cytochrome P450

EMB - Ethambutol

GlnA - Glutamine Synthetase

HDI - Herb–Drug Interaction

HDT - Host-Directed Therapy

HIV - Human Immunodeficiency Virus

INH - Isoniazid

MDR-TB - Multidrug-Resistant Tuberculosis

MRP - Multidrug Resistance-associated Protein
Mtb - *Mycobacterium tuberculosis*

NAT2 - N-acetyltransferase 2

NF-κB - Nuclear Factor kappa B

NTEP - National Tuberculosis Elimination Programme

OATP - Organic Anion Transporting Polypeptide

OATP1B1 - Organic Anion Transporting Polypeptide 1B1

OAT - Organic Anion Transporter

OCT - Organic Cation Transporter

P-gp - P-glycoprotein

PDB - Protein Data Bank

PknA - Protein Kinase A

PK - Pharmacokinetics

PZA - Pyrazinamide

RIF - Rifampicin

TB -Tuberculosis

UGT - Uridine 5-diphospho-glucuronosyltransferase

WHO - World Health Organization
WS - *Withania somnifera*

XDR-TB - Extensively Drug-Resistant Tuberculosis

Declarations

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Figures

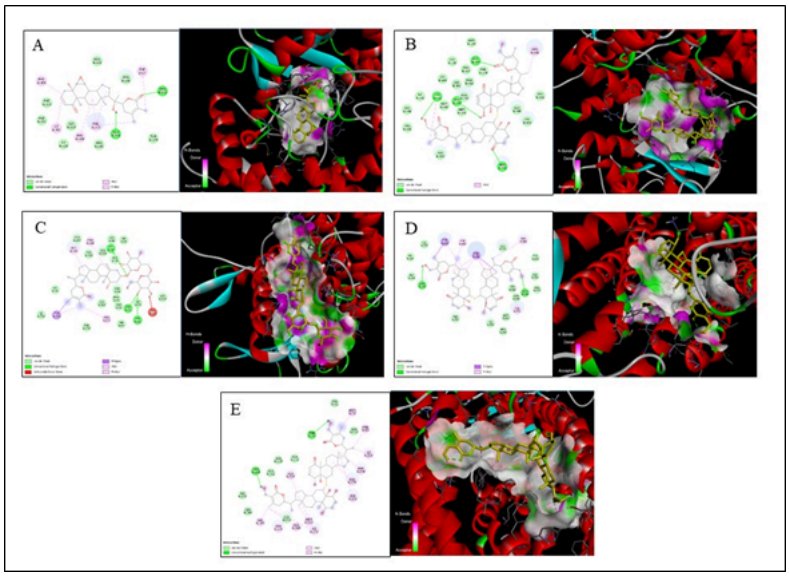


Figure 1

2D and 3D Interaction of representative WS phytoconstituents with the PK related targets

(A) interaction of Withanolide A with CYP3A4, (B) interaction of Ashwagandhanolide, with CYP2C19, (C) interaction of Withanoside V, with CYP2C9, (D) Interaction of Ashwagandhanolide, with OATP1B1, (E) Interaction of Ashwagandhanolide, with Pgp

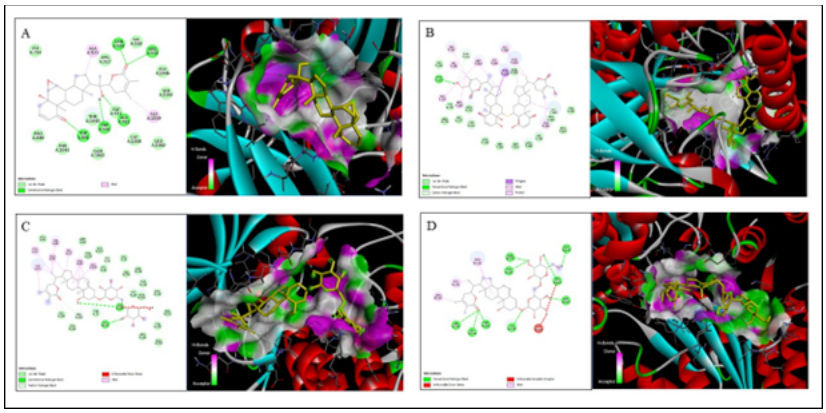


Figure 2

2D and 3D Interaction of representative WS phytoconstituents with Mtb bacterial targets

(A) Interaction of Withanolide A with Arabinosyl transferase C, (B) interaction of Ashwagandhanolide Mycobacterial glutamine synthetase, (C) Interaction Withanoside V with protein kinase A, (D) Interaction Withanoside V with Proteasomal ATPase

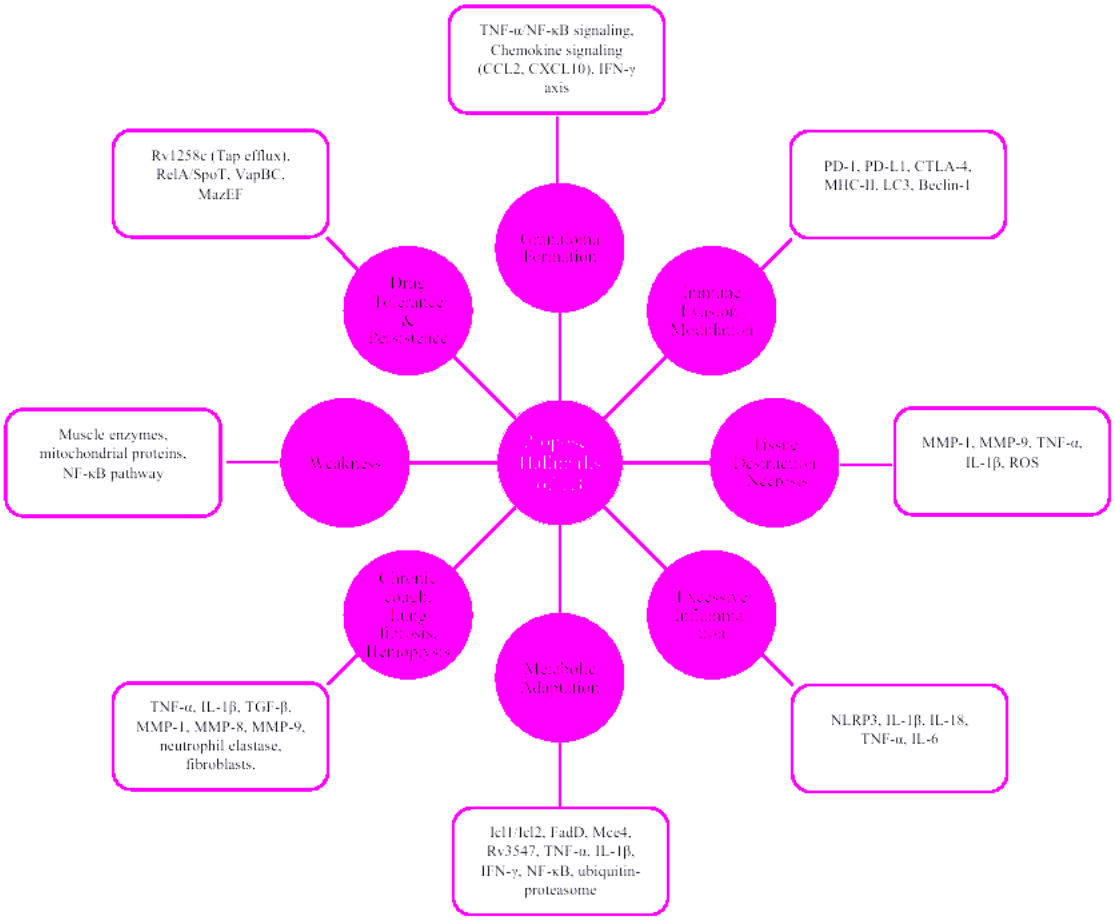


Figure 3

Proposed hallmarks of tuberculosis and their associated mechanistic pathways targeted by *Withania somnifera*.

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