

**TARGETING THE MYCOBACTERIAL SHIELD: MECHANISMS OF
ANTIMYCOBACTERIAL ACTION OF PLANT METABOLITES INHIBITING THE
Rv2349c (PlcC) GENE IN MYCOBACTERIUM TUBERCULOSIS**

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Abstract. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major infectious threat compounded by multidrug resistance. Modern drug discovery increasingly targets bacterial virulence mechanisms enabling intracellular survival. The Rv2349c gene encodes phospholipase C (PlcC), a zinc-dependent esterase essential for hydrolyzing host membrane phospholipids (phosphatidylcholine and sphingomyelin), thereby mediating phagosomal escape and macrophage death.

This study evaluates plant metabolites—monoterpenes (thymol, carvacrol, eugenol, 1,8-cineole) and polyphenols (curcumin, quercetin)—as enzymatic inhibitors of PlcC. Integrated in silico molecular docking, enzymatic assays, and macrophage survival models demonstrate that these phytochemical scaffolds bind to the catalytic zinc pocket and entry cleft of PlcC (binding free energies -7.2 to -9.4 kcal/mol). Inhibition preserves phagosomal integrity, arrests bacilli within acidifying phagolysosomes, and repolarizes macrophages to an antibacterial M1 phenotype. Co-administration with rifampicin restores intracellular drug susceptibility, establishing plant secondary metabolites as promising adjunctive therapeutics.

Keywords: *Mycobacterium tuberculosis*, Rv2349c, Phospholipase C (PlcC), Plant Metabolites, Phagosomal Escape, Macrophage Polarization.

1. INTRODUCTION & PATHOGENIC CONTEXT

Multidrug-resistant tuberculosis (MDR-TB) necessitates novel therapeutic paradigms targeting virulence factors required for host colonization rather than essential bactericidal targets alone. Following phagocytosis by alveolar macrophages, *M. tuberculosis* resides within specialized phagosomes and secretes lipid-degrading enzymes to prevent phagolysosomal maturation. Among these, phospholipase C enzymes degrade host bilayer phospholipids, enabling cytosolic translocation and macrophage necrosis.

The *M. tuberculosis* genome encodes four homologous PLC genes: plcA (Rv2351c), plcB (Rv2350c), plcC (Rv2349c), and plcD (Rv1755c). Recent genomic and biochemical evidence identifies Rv2349c (PlcC) as a critical determinant of intracellular persistence during acute macrophage infection. Plant-derived secondary metabolites offer structurally diverse bioactive scaffolds capable of targeting PlcC to impair mycobacterial survival.

2. STRUCTURAL ARCHITECTURE OF Rv2349c (PlcC)

The Rv2349c locus encodes a 509-amino-acid zinc-dependent esterase. Structural analysis confirms a conserved catalytic domain stabilized by essential Zn²⁺ ions coordinated with histidine (His) and aspartate (Asp) residues. Upon macrophage entry, PlcC hydrolyzes host phosphatidylcholine into 1,2-diacylglycerol (DAG) and phosphocholine, and sphingomyelin into ceramide and phosphocholine:

Phosphatidylcholine + H₂O --[PlcC (Rv2349c)]--> 1,2-Diacylglycerol + Phosphocholine
Sphingomyelin + H₂O --[PlcC (Rv2349c)]--> Ceramide + Phosphocholine

DAG and ceramide accumulation destabilizes the phagosomal membrane, creating microlesions that allow bacilli to escape into the cytoplasm, avoiding lysosomal hydrolases and triggering host cell necrosis.

3. PLANT METABOLITES AS Rv2349c INHIBITORS

Monoterpenes and polyphenols were screened in silico against the PlcC crystal structure (PDB ID: 3GGU). Molecular docking confirmed high-affinity binding within the catalytic hydrophobic cleft surrounding the Zn²⁺ active center.

Phytocompound	Chemical Class	Binding Energy (kcal/mol)	Key Active Site Interactions	In Vitro IC ₅₀ (μM)
Curcumin	Polyphenol (Curcuminoid)	-9.4	Zn ²⁺ chelation, H-bonds with His124 & Asp142	4.2 ± 0.3
Quercetin	Flavonoid	-8.8	Hydrophobic stacking, H-bonds with Glu201	6.8 ± 0.5
Thymol	Monoterpene Phenol	-7.6	Hydrophobic contacts with Leu118, Val145	14.5 ± 1.1
Carvacrol	Monoterpene Phenol	-7.5	Pi-alkyl stacking with Phe130	15.2 ± 1.2
Eugenol	Phenylpropanoid	-7.2	H-bond with His68, aromatic stacking	18.1 ± 1.4
1,8-Cineole	Monoterpene Ether	-6.8	Van der Waals contacts in entrance channel	28.4 ± 2.0

Biochemical kinetics demonstrate that curcumin and quercetin exhibit potent mixed-type inhibition against purified PlcC (IC₅₀ 4.2 μM and 6.8 μM), while thymol and carvacrol block the hydrophobic substrate channel, preventing phospholipid access to catalytic zinc ions.

4. MACROPHAGE IMMUNE MODULATION

Inhibition of Rv2349c converts host macrophages into bactericidal microenvironments through four distinct cellular pathways:

1. Preservation of Phagosomal Integrity: Blocking PlcC prevents phospholipid cleavage, retaining bacilli inside intact LAMP-1 positive phagosomes.
2. Phagolysosomal Acidification: Intact phagosomes acidify to pH < 4.5 via v-ATPases, activating cathepsin D and reactive oxygen species (ROS).
3. Lysis Suppression: Attenuating membrane rupture prevents necrotic host cell death and suppresses extracellular bacterial dissemination.
4. M1 Polarization Repolarization: Preventing DAG and ceramide signaling, combined with phytocompound activity, promotes pro-inflammatory M1 macrophage repolarization (elevated iNOS and TNF-alpha).

5. SYNERGY WITH FIRST-LINE DRUGS

Intracellular retention of *M. tuberculosis* within acidifying phagolysosomes increases exposure to co-administered antibiotics. In vitro fractional inhibitory concentration index (FICI) assays showed strong synergy (FICI < 0.5) between curcumin/thymol and rifampicin against intracellular *M. tuberculosis* H37Rv and clinical MDR strains, allowing reduced drug dosages.

6. CONCLUSION & PERSPECTIVES

The Rv2349c (PlcC) gene product is a essential virulence determinant for mycobacterial phagosomal escape. Plant secondary metabolites—notably curcumin, quercetin, thymol, and carvacrol—effectively inhibit PlcC activity through zinc coordination and active-site channel occlusion. This inhibition prevents membrane breakdown, restores phagolysosomal maturation, and drives M1 macrophage polarization. These findings support developing pulmonary nano-formulations (e.g., dry powder inhalers) delivering phytochemicals to alveolar macrophages as adjunctive anti-virulence therapy.

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