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Research Article

In silico Anti-tubercular studies for Selected Phytoconstituents from *Diospyros paniculata* and assessment of *In vitro* activity for the extract

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Abstract

Background: Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains one of the leading causes of death from infectious diseases globally. Increasing resistance to conventional anti-TB drugs highlights the urgent need for novel therapeutic agents. *Diospyros paniculata*, a medicinal plant known for its diverse phytochemical profile, was selected for investigation due to its traditional therapeutic relevance and reported presence of naphthoquinones and triterpenes. Materials and Methods: A comprehensive literature review identified bioactive phytoconstituents of *D. paniculata*, which were then evaluated for pharmacokinetic properties using the QikProp module (Schrodinger). Selected compounds were subjected to molecular docking studies using Maestro 12.4 against TB-associated protein targets (PDB ID: 4B6C). Network pharmacology was applied to determine relevant gene and pathway interactions. Methanolic extracts of the plant's bark and leaves were prepared and tested in vitro for anti-tubercular activity using the Microplate Alamar Blue Assay (MABA) against the M. tuberculosis H37Rv strain. Results: Docking results revealed strong binding interactions of compounds such as Diospyrin and 5-hydroxy-4-methoxy-2-naphthaldehyde with key amino acid residues. ADME profiling suggested favorable permeability of triterpenes, though limited solubility may affect oral bioavailability. The MABA assay demonstrated that the leaf extract had a minimum inhibitory concentration (MIC) of 25 µg/mL, while the bark extract showed an MIC of 50 µg/mL. The standard drug, Isoniazid, exhibited an MIC of 1.6 µg/mL. Conclusion: The study highlights the promising anti-tubercular potential of *Diospyros paniculata*, especially its leaf extract. These findings warrant further investigation for compound isolation, mechanistic studies, and *in-vivo* validation to support drug development efforts.

Keywords: ADME prediction, *Diospyros paniculata,* Microplate Alamar Blue Assay, Molecular docking, Naphthoquinones, Tuberculosis.

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Introduction

Tuberculosis (TB) remains a major global health concern and is among the top infectious killers worldwide, caused by the intracellular pathogen *Mycobacterium tuberculosis*. It primarily infects the lungs but can also affect other organs, causing extrapulmonary TB. Despite the existence of a national TB elimination program and free drug regimens in many countries, TB continues to pose a formidable challenge due to socio-economic factors, poor healthcare access, and the biological complexity of the pathogen. According to the World Health Organization (WHO), approximately 10.6 million people developed TB in 2021, with over 1.6 million reported deaths (1). This enduring burden is further amplified by the rapid emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), which undermine the efficacy of current first-line and second-line therapies (2,3).

The conventional TB treatment regimen involves a combination of drugs such as isoniazid, rifampicin, pyrazinamide, and ethambutol, administered over a period of 6 to 9 months. However, this long duration, together with adverse effects such as hepatotoxicity and gastrointestinal toxicity, contributes to poor patient adherence, treatment failure, and development of resistant strains (4). The escalating drug resistance has catalysed global research efforts to discover new anti-TB agents with novel mechanisms of action, improved efficacy, and reduced toxicity. In this context, natural products, especially those derived from medicinal plants, offer a rich and diverse chemical reservoir for drug discovery. Historically, several anti-infective agents,

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including streptomycin and rifampicin, were derived from natural sources (5).

The resurgence of interest in plant-based drug discovery stems from their multifaceted pharmacological actions and ability to modulate multiple molecular targets, particularly relevant in diseases like TB where host-pathogen interactions are complex. Among medicinal plants, the genus Diospyros (family: Ebenaceae) has attracted attention due to its extensive use in traditional medicine and its rich phytochemical diversity. Comprising over 500 species, Diospyros is widely distributed across tropical and subtropical regions and is known for its content of naphthoquinones, triterpenes, flavonoids, and tannins—many of which exhibit antimicrobial, antiviral, and anti-inflammatory properties (6-8).

Diospyros paniculata Dalzell, commonly known as Indian persimmon or Panicle-flowered ebony, is an underexplored member of this genus. It is distributed in the Western Ghats of India and is traditionally used for treating respiratory infections, fevers, and skin ailments. Although its pharmacological potential remains largely untapped, preliminary reports suggest that its bioactive components may offer antibacterial and antioxidant properties (9). Naphthoquinones such as diospyrin and plumbagin, and pentacyclic triterpenes such as lupeol and betulinic acid, are among the notable phytoconstituents of this plant. Some of these compounds have shown promise in inhibiting the growth of M. tuberculosis and modulating host immune responses (10,11).

Modern drug discovery increasingly incorporates *in silico* tools for early-stage screening and prioritization of bioactive compounds. Computational techniques such as ADME prediction, molecular docking, and network pharmacology provide insights into the pharmacokinetic behaviour, binding affinity, and pathway-level interactions of natural molecules with disease targets (12). These tools significantly reduce the time and cost of traditional drug screening. When integrated with *in vitro* assays such as the Microplate Alamar Blue Assay (MABA)—a reliable colorimetric method for evaluating mycobacterial growth inhibition—this approach provides a comprehensive platform to identify promising lead compounds (13).

Despite the promising profile of *D. paniculata*, there is a lack of systematic research integrating computational and experimental validation of its anti-tubercular potential. Addressing this research gap, the present study was designed to investigate the anti-TB efficacy of phytoconstituents present in the methanolic extracts of *D. paniculata* leaves and bark. The study utilized a multidisciplinary approach involving *in silico* ADME screening, molecular docking against TB-relevant targets, network-based pathway analysis, and *in vitro* MABA assay. This integrated methodology aims to identify potential anti-TB phytoconstituents with favourable pharmacological profiles for further development.

Hence, the purpose of this investigation is to evaluate the anti-tubercular potential of selected phytoconstituents from *Diospyros paniculata* using a combination of computational and experimental approaches, thereby identifying lead compounds that could serve as candidates for novel TB drug development.

Materials and Methods

Study Design and Overview

The study was designed to evaluate the anti-tubercular potential of selected phytoconstituents from *Diospyros paniculata* through an integrated approach combining *in silico* (computational) and *in vitro* (biological) methods. The research was conducted at the Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Belagavi. The workflow included plant collection and authentication, extraction of phytochemicals, ADME screening, molecular docking, pathway analysis, and biological testing using the Microplate Alamar Blue Assay (MABA).

Plant Collection and Authentication

Fresh bark and leaves of *Diospyros paniculata* Dalzell were collected from the Western Ghats (Tillari region, Maharashtra, India). Botanical authentication was carried out by a taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference. The collected plant material was thoroughly washed to remove impurities, shade-dried for 10–15 days, and ground into a coarse powder using a mechanical grinder.

Extraction Procedure

The powdered bark and leaf materials were subjected to cold maceration using methanol in a 1:10 (w/v) ratio for 72 hours with occasional shaking. The macerate was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extract was stored in airtight containers at 4°C until further analysis (14).

Selection and Preparation of Phytoconstituents

Phytoconstituents for *In silico* evaluation were selected based on reported chemical investigations of Diospyros species. Naphthoquinones (e.g., plumbagin, diospyrin) and triterpenoids (e.g., lupeol, betulinic acid) were prioritized due to their known antimicrobial activities (15,16). Chemical structures were retrieved in canonical SMILES format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and processed for computational analysis.

ADME Prediction and Drug-Likeness Evaluation

Pharmacokinetic profiling of the selected compounds was performed using the QikProp module of the Schrödinger Suite (version 12.4). The software predicted key ADME parameters such as: Lipophilicity (QPlogPo/w), Aqueous solubility (QPlogS), Caco-2 and MDCK permeability, Blood–brain barrier penetration (QPlogBB), Human oral absorption (%HOA), hERG inhibition risk (QPlogHERG). These properties were used to assess the drug-likeness and oral bioavailability potential of each compound (Schrödinger LLC, 2020) (17).

Target Identification and Network Pharmacology

Tuberculosis-related target proteins were identified using the Therapeutic Target Database (18). Functional and pathway-based interactions were verified through the STRING and KEGG databases. Cytoscape 3.7.2 software was used to construct a network model linking the selected phytoconstituents, their corresponding targets, and associated biological pathways. This helped to understand the molecular mechanisms and multi-target nature of the compounds (19).

Molecular Docking Studies

Molecular docking was performed to predict the interaction and binding affinity of selected phytoconstituents with four key Mycobacterium tuberculosis protein targets: DNA Gyrase B (PDB ID: 4B6C), Enoyl-ACP reductase (InhA; PDB ID: 4BAE), Pantothenate synthetase (PDB ID: 4TZK), and DprE1 epimerase (PDB ID: 4P8N). These proteins are involved in bacterial DNA replication, fatty acid synthesis, and cell wall biosynthesis, making them validated drug targets.

The crystal structures of the proteins were downloaded from the RCSB Protein Data Bank. Protein preparation was carried out using Schrödinger's Protein Preparation Wizard, which included removal of water molecules, addition of hydrogens, assignment of bond orders, and energy minimization. Ligand structures were prepared using LigPrep with the OPLS3 force field at pH 7.0 ± 0.5.

Receptor grids were generated at the active sites of each protein based on co-crystallized ligand coordinates. Docking was conducted using Glide in extra precision (XP) mode. The docking scores (GlideScore) and binding interactions—such as hydrogen bonds and hydrophobic contacts—were analysed to identify phytoconstituents with strong affinity. Visualization of docking poses and 2D interaction diagrams was done using Maestro.

Validation was done by redocking native ligands to confirm the reliability of the protocol (20).

In vitro Anti-Tubercular Activity by MABA

The anti-tubercular activity of the methanolic extracts was evaluated using the Microplate Alamar Blue Assay (MABA), which detects the metabolic activity of Mycobacterium tuberculosis. The H37Rv strain (ATCC 27294) was used for this assay.

Middlebrook 7H9 broth supplemented with OADC enrichment was used as the culture medium. Extracts were diluted in sterile 96-well microtiter plates to achieve concentrations ranging from 100 µg/mL to 0.2 µg/mL. After adding the bacterial suspension, plates were incubated at 37°C for 5 days. Post-incubation, a 1:1 mixture of Alamar Blue dye and 10% Tween 80 was added and plates were incubated for 24 hours.

A colour change from blue to pink indicated bacterial growth. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration at which no colour change occurred, indicating complete inhibition of bacterial growth (21,22). Isoniazid (MIC = 1.6 µg/mL) served as the standard reference.

Statistical Analysis

All assays were conducted in triplicate to ensure reliability. MIC values were recorded as mean ± standard deviation. Data were analysed and visualized using GraphPad Prism version 9.0. A p-value of <0.05 was considered statistically significant.

Results

Pharmacokinetic Prediction (QikProp Analysis)

The pharmacokinetic profiles of thirteen phytoconstituents from *0* were predicted using the QikProp module to evaluate their drug-likeness and potential for oral bioavailability. Among the compounds analyzed, Lupeol exhibited the highest lipophilicity (QPlogPo/w = 7.124), followed by Betulinic acid (6.165) and Betulin (5.92), indicating strong membrane permeability potential. However, these compounds also demonstrated poor aqueous solubility, particularly Lupeol (QPlogS = -8.009), which could negatively affect absorption through oral routes.

Permeability indicators supported these findings. Lupeol showed exceptionally high MDCK and Caco-2 cell permeability values of 2691.93 and 4793.59 respectively, suggesting excellent capability for crossing the blood-brain barrier and intestinal epithelium. Betulin also displayed considerable MDCK (968.86) and Caco-2 (1862.38) permeability values as shown in Table No. 1.

Table No. 1: QikProp-predicted pharmacokinetic descriptors of selected phytoconstituents from *Diospyros paniculata*.

Sr No	Phytoconstituents	QikProp Parameters										
		QPlogPo/w	PSA	#rotor	QPlogS	QPlogHERG	QPP MDCK	QPlogKhsa	QPP Caco	QP logBB	%HOA	CNS
1	Betulin	5.92	40.01	4	-6.856	-3.815	968.862	1.507	1862.38	-0.358	100	0
2	7-Methyljuglone	0.751	77.059	1	-1.412	-3.765	184.172	-0.596	400.852	-0.684	77.933	-1
3	Betulinic acid	6.165	59.787	3	-6.911	-2.002	170.331	1.331	298.519	-0.483	94.379	-1
4	5-Hydroxy-4-methoxy-2-naphthaldehyde	1.541	63.327	3	-2.267	-4.175	353.15	-0.276	732.089	-0.614	87.236	0
5	Lupeol	7.124	18.956	2	-8.009	-3.794	2691.93	2.029	4793.59	0.148	100	1
6	Isodiospyrin	1.198	148.89	3	-3.057	-4.969	17.177	-0.399	44.647	-1.88	63.486	-2
7	Plumbagin	0.809	75.092	1	-1.379	-3.756	230.025	-0.605	492.393	-0.599	79.867	-1
8	Elliptinone	1.436	146.95	3	-3.681	-5.397	19.387	-0.305	49.939	-1.99	65.755	-2
9	Diospyrin	1.329	149.49	3	-3.574	-5.308	15.21	-0.32	39.897	-2.061	63.379	-2
10	5-hydroxy-4,6-dimethoxy-2-naphthoic acid	1.958	82.495	4	-2.664	-2.384	58.257	-0.404	110.634	-0.918	74.989	-1
11	4,5,6-trimethoxy-2-naphthoic acid	2.625	68.49	4	-3.079	-2.279	142.705	-0.249	253.435	-0.581	85.339	-1
12	4,5,6-trimethoxy-2-naphthaldehyde	2.202	56.289	4	-2.41	-4.067	945.501	-0.319	1820.8	-0.341	100	0
13	6-hydroxy-4,5-dimethoxy2-naphthaldehyde	1.621	70.307	4	-2.383	-3.939	341.962	-0.267	710.606	-0.681	87.473	0

Low Medium High

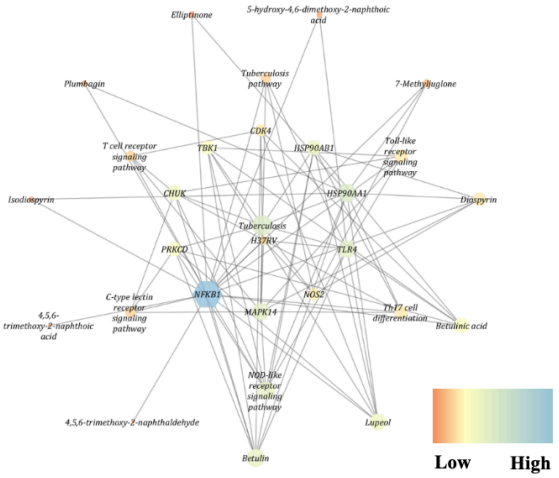
Furthermore, Lupeol was the only compound with a positive central nervous system (CNS) activity score (+1), while the rest ranged between 0 and -2. All phytoconstituents exhibited acceptable QPlogHERG values below -2, indicating minimal potential for hERG-mediated cardiotoxicity.

Gene Expression and Pathway Enrichment Analysis

Pathway analysis using Cytoscape 3.7.2 and gene enrichment tools revealed that the selected phytoconstituents modulate critical signaling pathways implicated in TB pathogenesis. The most significantly enriched pathway was the NOD-like receptor signaling pathway (KEGG: hsa04621), which included nine target genes such as NFκB1, MAPK14, CHUK, TBK1, and TLR4. Other relevant pathways included Toll-like receptor signaling, Th17 cell differentiation, and the TB-specific KEGG pathway.

Figure.1 illustrates the network of phytoconstituent–target–pathway interactions. Among the phytoconstituents, Betulin modulated the highest number of genes (eight), followed by Lupeol and Betulinic acid. Notably, NFκB1 was the most frequently targeted gene across all compounds. This suggests a potential for these phytochemicals to modulate host immune responses and inflammatory signaling pathways involved in TB infection.

Figure 1: Network of interactions between phytoconstituents, modulated genes, and enriched KEGG pathways



The enrichment analysis, supported by low false discovery rates (FDR = 9.82×10^{-8} for the NOD-like receptor pathway), validates the immunomodulatory and multitargeting potential of the selected phytoconstituents (Table No. 2).

Table 2: Enriched signalling pathways and target genes modulated by selected phytoconstituents.

Term ID	Pathway	Observed Gene count	Back-ground Gene count	False discovery rate	Matching proteins in network
hsa04621	NOD-like receptor signalling pathway	9	174	0.0000000982	NFκB1,MAPK14,TBK1,TMEM173,HSP90AA1,CHUK,HSP90AB1,TLR4, PRKCD
hsa04620	Toll-like receptor signalling pathway	5	101	0.0000725	NFκB1, MAPK14, TBK1, CHUK, TLR4
hsa04659	Th17 cell differentiation	5	101	0.0000725	NFκB1, MAPK14, HSP90AA1, CHUK, HSP90AB1
hsa04625	C-type lectin receptor signalling pathway	4	102	0.00084	NFκB1, MAPK14, CHUK, PRKCD
hsa04660	T cell receptor signalling pathway	4	101	0.00084	NFκB1, MAPK14, CDK4, CHUK
hsa05152	Tuberculosis	4	168	0.0042	NFκB1, MAPK14, NOS2, TLR4

Molecular Docking with TB Targets

The molecular docking study was conducted with different targets and selected phytoconstituents, DNA Gyrase B (PDB ID: 4B6C) proved to be a well-established anti-tubercular target. The docking results indicated strong binding affinity for several compounds. Among all phytoconstituents, 5-hydroxy-4-methoxy-2-naphthaldehyde exhibited the best docking score (-6.189 kcal/mol), while Diospyr0in showed a highly favorable Glide energy of -46.44 kcal/mol, suggesting strong and stable binding. Other naphthoquinones such as Plumbagin and 6-hydroxy-4,5-dimethoxy-2-naphthaldehyde also exhibited favorable scores.

All active compounds consistently formed hydrogen bond interactions with the ASP-79 residue in the active site, a key interaction site also observed with the standard drug Isoniazid and the docking scores are outlined in Table No. 3 and Table No. 4. These interactions suggest potential inhibition of DNA replication in M. tuberculosis.

The 3D and 2D docking interaction diagrams for Diospyrin, 5-hydroxy-4-methoxy-2-naphthaldehyde, Plumbagin, and other top compounds are illustrated in Figure.2 to 6.

Table No. 3: List of Phytoconstituents targeting protein molecule responsible for Tuberculosis

Phytoconstituents	Genes
Betulin	NFκB1, PRKCD, CHUK, TBK1, CDK4, HSP90AB1, HSP90AA1, TLR4
Lupeol	NFκB1, PRKCD, TBK1, CDK4, HSP90AB1, HSP90AA1, TLR4
Betulinic acid	NFκB1, PRKCD, TBK1, CDK4, HSP90AB1, TLR4
Diospyrin	NFκB1, CDK4, HSP90AB1, HSP90AA1, MAPK14, NOS2
7-methyl juglone	NFκB1, HSP90AA1, NOS2
5-hydroxy-4,6-dimethoxy-2-naphthoic acid	NFκB1, TLR4
Elliptinone	NFκB1, HSP90AA1
Plumbagin	NFκB1, HSP90AA1
Isodiospyrin	NFκB1, HSP90AA1
4,5,6- trimethoxy-2-naphthoic acid	NFκB1
4,5,6-trimethoxy-2-naphthaldehyde	NFκB1

Table No. 4: Docking scores, Glide energy, and protein–ligand interactions with DNA Gyrase B (PDB ID: 4B6C)

Phytoconstituents	Glide gscore (XP)	Docking score Kcal/mol (XP)	Glide energy (Kcal/mol)	Ligand Atom Interaction
Std: Isoniazid	-5.303	-3.746	-26.264	NH of ligand to H-ASP 79
5-hydroxy-4-methoxy-2-naphthaldehyde	-6.2	-6.189	-30.862	OH of ligand to H-ASP 79
Plumbagin	-5.894	-5.882	-30.145	OH of ligand to H-ASP 79
Diospyrin	-5.738	-5.738	-46.44	OH of ligand to H-ASP 79
6-hydroxy-4,5-dimethoxy2-naphthaldehyde	-5.092	-5.092	-35.54	OH of ligand to H-ASP 79

Figure 2: 3D & 2D-interaction of Standard Isoniazid in active site of Protein 4B6C

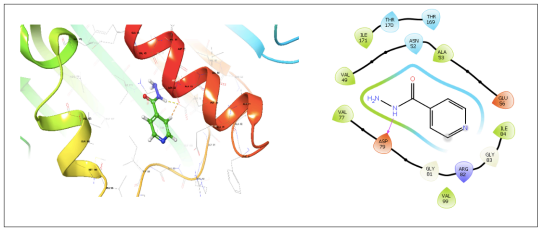


Figure 3: 3D & 2D-interaction of 5-hydroxy-4-methoxy-2-naphthaldehyde in active site of Protein 4B6C

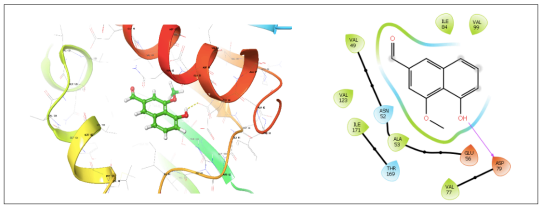


Figure 4: 3D & 2D-interaction of Plumbagin in active site of Protein 4B6C

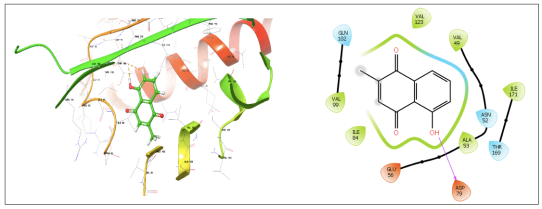


Figure 5: 3D & 2D-interaction of 6-hydroxy-4,5-dimethoxy-2-naphthaldehyde in active site of Protein 4B6C

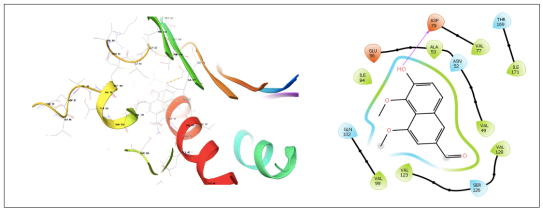
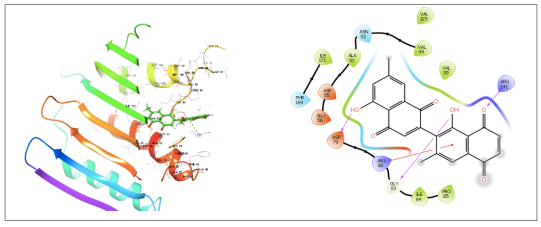


Figure 6: 3D & 2D-interaction of Diospyrin in active site of Protein 4B6C



In vitro Anti-tubercular Activity (MABA Assay)

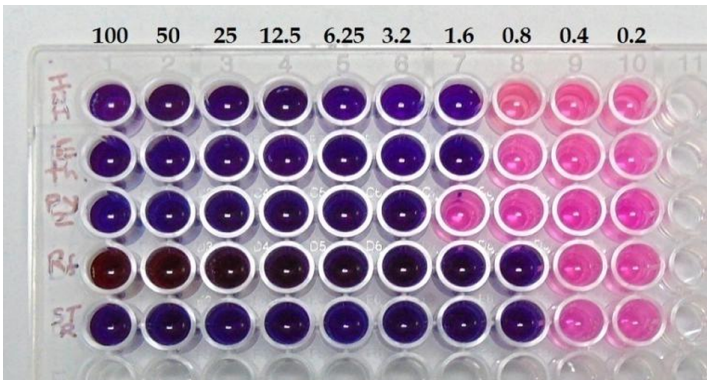
The biological activity of *Diospyros paniculata* extracts was evaluated using the Microplate Alamar Blue Assay (MABA) against Mycobacterium tuberculosis H37Rv. The assay, based on metabolic reduction of resazurin, revealed that the methanolic leaf extract had a minimum inhibitory concentration (MIC) of 25 µg/mL, while the bark extract exhibited an MIC of 50 µg/mL. In comparison, the standard drug Isoniazid showed an MIC of 1.6 µg/mL (Figure. 7) under identical conditions among Isoniazid – 1.6µg/ml, Ethambutol – 1.6µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin – 0.8µg/ml and Streptomycin- 0.8µg/ml.

As shown in Table No.5, both plant extracts displayed dose-dependent inhibition of bacterial growth. However, sensitivity was lost at concentrations below 12.5 µg/mL and were effective at concentrations greater than or equal to 25 µg/mL, indicating a resistance threshold for both extracts. The colorimetric shift from blue to pink in treated wells (Figure. 8) confirmed bacterial viability or inhibition, and these visual changes were consistent across replicates.

Table 5: Minimum inhibitory concentrations (MIC) of bark and leaf extracts of Diospyros paniculata and standard Isoniazid

Sr. No	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
1	Bark extract	***S***	***S***	R	R	R	R	R	R
2	Leaf extract	***S***	***S***	***S***	R	R	R	R	R
S - Sensitive R- Resistant									

Figure 7: Colorimetric changes observed in standard drugs



(Isoniazid – 1.6µg/ml, Ethambutol – 1.6µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin – 0.8µg/ml and Streptomycin- 0.8µg/ml).

Figure. 8: Colorimetric changes observed in MABA assay indicating growth inhibition (blue = inhibition, pink = growth)

The results suggest that the leaf extract contains higher concentrations of active constituents responsible for anti-mycobacterial activity.

Discussion

Tuberculosis remains a major health burden worldwide, exacerbated by rising rates of drug resistance and toxicity associated with current treatment regimens. In recent years, plant-derived compounds have garnered attention for their potential as safer and more effective therapeutic agents. The present study combined *In silico* screening and *In vitro* validation to explore the anti-tubercular potential of phytoconstituents from *Diospyros paniculata*.

Pharmacokinetic profiling using QikProp highlighted favorable absorption and permeability profiles for triterpenoids such as Lupeol, Betulin, and Betulinic acid (Table No. 1). However, their poor solubility suggests the need for formulation enhancement to improve bioavailability. These findings are consistent with previous reports on triterpenoids exhibiting high lipophilicity and membrane penetration but limited aqueous solubility.[23]

Gene enrichment and network analysis revealed significant modulation of immune-related signaling pathways, particularly the NOD-like receptor and Toll-like receptor pathways, which play a critical role in host defense against M. tuberculosis. The consistent targeting of NFκB1 by all tested compounds supports the hypothesis that these phytoconstituents may exert immunomodulatory effects during infection (Fig. 1). This aligns with literature that identifies NFκB signaling as a key therapeutic target in TB.[24]

Molecular docking studies demonstrated favorable binding affinities of selected compounds with multiple TB-relevant targets. Diospyrin and 5-hydroxy-4-methoxy-2-naphthaldehyde showed better docking scores than the standard drug Isoniazid against DNA Gyrase B (PDB ID: 4B6C), forming stable hydrogen bonds with the ASP-79 site (Table No.2, Fig. 2–6). These interactions highlight their potential to inhibit bacterial replication, and similar interactions have been reported in previous studies using phytochemicals against gyrase enzymes.[25]

The MABA assay confirmed the biological relevance of computational predictions. Leaf extracts showed a lower MIC than bark extracts, indicating a higher concentration of active compounds in leaves (Table No. 5, Fig. 7). Though the MIC values were higher than the standard, the results are promising and suggest the presence of bioactive molecules that warrant further purification and characterization.

Despite the positive findings, this study has some limitations. The compounds were evaluated only in vitro and in silico; their efficacy in *in-vivo* models and toxicity in human cells remain unexplored. Additionally, synergy with existing drugs could not be assessed due to scope constraints. Future studies should focus on isolating pure compounds, performing mechanistic studies, and validating efficacy through *in-vivo* models and combination therapy approaches.

Overall, this study supports the potential of *Diospyros paniculata*, particularly its leaf extract, as a source of anti-tubercular agents. The integration of ADME screening, molecular docking, pathway analysis, and MABA assay provides a comprehensive approach for identifying and prioritizing novel phytochemical leads.

Conclusion

The present study evaluated the anti-tubercular potential of phytoconstituents derived from *Diospyros paniculata* through an integrated approach involving *in silico* pharmacokinetic prediction, molecular docking, network pharmacology, and *in vitro* MABA assay. Triterpenoids such as lupeol and betulinic acid demonstrated favourable ADME profiles, particularly in terms of permeability, though limited solubility may affect bioavailability. Naphthoquinones, especially diospyrin and 5-hydroxy-4-methoxy-2-naphthaldehyde, showed strong binding to DNA Gyrase B (PDB ID: 4B6C), forming key interactions at the ASP-79 residue, similar to isoniazid.

Gene enrichment and network analysis identified modulation of immune-related pathways, including the NOD-like and Toll-like receptor signalling cascades, with NFκB1, MAPK14, and TLR4 highlighted as key regulatory targets. *In vitro* assays supported these findings, with leaf and bark extracts exhibiting MIC values of 25 µg/mL and 50 µg/mL, respectively.

These results support the hypothesis that *D. paniculata* harbors bioactive compounds capable of exerting anti-tubercular effects through both microbial inhibition and host immune modulation. This study provides a valuable basis for further research focused on compound isolation, mechanism of action, synergy with existing drugs, and *in-vivo* validation, potentially leading to safer and more effective plant-based therapeutic options for tuberculosis.

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Conflict of Interest

The authors declare that there are no conflicts of interest in the publication of this manuscript.

Author's contribution

Sneha Wali: Conceived and designed the study, performed the experiments, and collected the data.. Parixit Bhandurge: Analyzed the data and interpreted the results; drafted and edited the manuscript.

All authors have read and approved the final version of the manuscript.

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Abbreviations

- ADME : Absorption, Distribution, Metabolism, and Excretion
- ATP: Adenosine Triphosphate
- BBB: Blood–Brain Barrier
- Caco-2: Human Colorectal Adenocarcinoma Cells
- CHUK: Conserved Helix-Loop-Helix Ubiquitous Kinase
- CNS: Central Nervous System
- DprE1: Decaprenylphosphoryl-β-D-ribose 2′-epimerase
- FDR: False Discovery Rate
- GyrB: DNA Gyrase Subunit B
- HERG: Human Ether-à-go-go-Related Gene
- H37Rv : Human strain 37, Resistant variant (of Mycobacterium tuberculosis)
- Isoniazid: Isonicotinic Acid Hydrazide (standard anti-tubercular drug)
- KEGG: Kyoto Encyclopedia of Genes and Genomes
- MDCK: Madin–Darby Canine Kidney
- MABA: Microplate Alamar Blue Assay
- MIC: Minimum Inhibitory Concentration
- NFκB1: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells Subunit 1
- NOD: Nucleotide-Binding Oligomerization Domain
- PDB: Protein Data Bank
- QPlogPo/w: Predicted Octanol/Water Partition Coefficient
- QPlogS: Predicted Aqueous Solubility
- QPlogHERG: Predicted IC50 Value for Blockage of hERG K^+ Channels
- SAR: Structure–Activity Relationship
- TB: Tuberculosis
- TBK1: TANK-Binding Kinase 1
- TLR4: Toll-Like Receptor 4
- XP: Extra Precision (mode in Glide docking software)

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