

In-Silico Studies, Phytochemical Characterization and In-Vitro Anti-Tubercular Evaluation of *Barleria prionitis* Leaf Ethanolic Extract

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Abstract: Tuberculosis (TB) remains a major public-health problem, and drug resistance sustains the search for new antimycobacterial leads, including those from medicinal plants. The present study evaluated the ethanolic leaf extract of *Barleria prionitis* Linn. (EEBP; Acanthaceae) by phytochemical, chromatographic, computational and microbiological approaches. Leaves collected in Theni district, Tamil Nadu, were extracted with ethanol in a Soxhlet apparatus. Preliminary screening showed alkaloids, carbohydrates, glycosides, sterols, flavonoids, phenols, terpenoids and tannins. Thin-layer chromatography indicated phenolic, terpenoid and steroidal constituents, and GC-MS analysis resolved 60 peaks. Spectrophotometric estimation gave 22.57 mg ursolic acid equivalents/g (terpenoids), 24.62 mg cholesterol equivalents/g (sterols) and 25.04 mg gallic acid equivalents/g (phenols) of extract. Six GC-MS constituents were docked against the *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (InhA) using AutoDock. Epilupeol (−9.4 kcal/mol) and γ -sitosterol (−9.3 kcal/mol) gave the strongest predicted binding, followed by 2-methoxy-4-vinylphenol (−7.0), diethyl phthalate (−6.9), 1,2,3-benzenetriol (−6.3) and n-hexadecanoic acid (−6.2). Both top-scoring ligands exceeded Lipinski's logP limit, so their oral drug-likeness is doubtful. In the microplate Alamar Blue assay against *M. tuberculosis* H37Rv (ATCC 27294), EEBP showed a minimum inhibitory concentration (MIC) of 25 μ g/mL, which is eight-fold higher than that of pyrazinamide (3.125 μ g/mL) in the same assay. EEBP therefore shows moderate in-vitro antimycobacterial activity and merits bioassay-guided isolation of its triterpenoid and sterol constituents; the docking predictions are hypotheses that require enzymatic and cellular validation.

Keywords: *Barleria prionitis*; anti-tubercular activity; GC-MS; molecular docking; InhA; microplate Alamar Blue assay.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the leading infectious causes of death worldwide, and India carries the largest national share of the global burden, accounting for roughly a quarter of incident cases. Treatment is further complicated by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains and by HIV co-infection [1]. New chemical entities with novel scaffolds or mechanisms are therefore needed.

Medicinal plants are a long-standing source of antimycobacterial leads. Crude extracts of several traditionally used plants have shown in-vitro activity against *M. tuberculosis* and related mycobacteria [2,3], and pentacyclic triterpenoids

from Argentine and Chilean plants have been reported to inhibit the bacillus [4].

Barleria prionitis Linn. (Vajradanti; family Acanthaceae) is a spiny shrub used in Ayurvedic and folk medicine for oral and inflammatory conditions, fever and other ailments [5]. It contains iridoid and phenylethanoid glycosides, flavonoids, phenolic acids, and triterpenoids and sterols such as lupeol [5,6]. The present work examines the leaf of this plant as a source of antimycobacterial constituents.

InhA, the NADH-dependent enoyl-acyl carrier protein reductase of the mycobacterial FAS-II system, catalyses a late step of mycolic acid elongation and is the validated target of isoniazid [7]. Molecular docking offers a rapid way to prioritise

phytoconstituents that may bind such a target before isolation and enzyme assays [8]. We therefore combined (i) phytochemical and chromatographic characterisation of the ethanolic leaf extract (EEBP), including GC-MS profiling; (ii) docking of selected GC-MS constituents against InhA with

likeness assessment; and (iii) determination of the MIC of EEBP against *M. tuberculosis* H37Rv by the microplate Alamar Blue assay (MABA).

2. Materials and Methods

2.1 Plant material

Leaves of *B. prionitis* were collected from Sirumalai, Theni district, Tamil Nadu, India, in June 2026. The plant was authenticated by Dr. Stephen, Department of Botany (retired), American College. The leaves were shade-dried, powdered in a mixer, sieved and stored in an airtight container.

2.2 Preparation of the extract

Powdered leaf (50 g) was extracted with 500 mL of ethanol in a Soxhlet apparatus at 60–80 °C. The extract was filtered, and the solvent was evaporated at room temperature to obtain the ethanolic extract (EEBP).

2.3 Preliminary phytochemical screening

EEBP was screened by standard qualitative procedures for alkaloids (Mayer's, Dragendorff's and Hager's tests), carbohydrates (Molisch's, Fehling's and Benedict's tests), glycosides (Borntrager's and ferric chloride tests), sterols (Liebermann–Burchard test), flavonoids (Shinoda, alkali, lead acetate and acid tests), phenols (ferric chloride, lead acetate and ellagic acid tests), terpenoids (Salkowski test) and tannins (phenazone and gelatin tests).

2.4 Thin-layer chromatography

Phenolics were resolved with acetic acid–chloroform (1:9) and detected with 10% ferric chloride, with gallic acid as reference. Terpenoids were resolved with chloroform–methanol (9:1) and steroids with chloroform–methanol (95:5); both were detected with anisaldehyde–sulphuric acid spray. Retention factors (R_f) were calculated for the resulting spots.

2.5 Quantitative estimation of phytoconstituents

Total terpenoids. The vanillin–sulphuric acid method was used. Extract solution (0.5 mL) was mixed with 0.5 mL of 5% vanillin in glacial acetic acid and 2.5 mL of 70% sulphuric acid in glacial acetic acid, heated at 60 °C for 15 min and cooled, and the absorbance was read at 538 nm against a reagent blank. Ursolic acid (10–50 µg/mL) was the standard; results are expressed as mg ursolic acid equivalents (UAE) per g of dry extract.

Total sterols. The Liebermann–Burchard reaction was used. A chloroform solution of the extract (2 mL) was treated with 2 mL of acetic anhydride and 2 mL of concentrated sulphuric acid, kept at room temperature for 30 min, and the absorbance was read at 620 nm. Cholesterol (10–50 µg/mL in chloroform) was the standard; results are expressed as mg cholesterol equivalents (CE) per g of dry extract.

Total phenolics. The Folin–Ciocalteu method [9] was used with 1 N Folin–Ciocalteu reagent and 10% sodium carbonate; absorbance was read at 760 nm on a Shimadzu UV-1800 spectrophotometer. Gallic acid (10–50 µg/mL) was the standard; results are expressed as mg gallic acid equivalents (GAE) per g of dry extract.

2.6 GC-MS analysis

EEBP was analysed on a PerkinElmer Clarus 680 gas chromatograph coupled to a Clarus 600 (EI) mass spectrometer (TurboMass 5.4.2) fitted with an Elite-5MS fused-silica column (30 m × 0.25 mm i.d., 0.25 µm film; 5% biphenyl–95% dimethylpolysiloxane). Helium was the carrier gas at 1 mL/min. The injector was held at 260 °C, and 1 µL was injected with a 10:1 split. The oven was held at 60 °C for 2 min, raised at 10 °C/min to 300 °C and held for 6 min (total run 32 min). Transfer-line and ion-source temperatures were 210°C; ionisation was by electron impact at 70 eV, with a solvent delay of 1 min and scanning from 40 to 600 Da (scan time 0.2 s, interval 0.1 s). Compounds were tentatively identified by comparing their mass spectra with the NIST 2008 library [10].

2.7 In-silico studies

Docking. The crystal structure of *M. tuberculosis* InhA (PDB ID) was obtained from the RCSB Protein Data Bank. Unwanted chains, the co-crystallized ligand and water molecules were removed in Discovery Studio Visualizer; polar hydrogens and Kollman charges were added in AutoDockTools 1.5.6. Six GC-MS constituents (epilupeol, γ -sitosterol, n-hexadecanoic acid, diethyl phthalate, 1,2,3-benzenetriol and 2-methoxy-4-vinylphenol) were drawn in ChemSketch, converted to 3D PDB format through SMILES, and prepared with Gasteiger charges and rotatable-bond detection. A $60 \times 60 \times 60$ grid box was generated with AutoGrid, and docking

was run in AutoDock 4 with the Lamarckian genetic algorithm and default search parameters [11]. The top-ranked pose of each ligand was inspected in Discovery Studio Visualizer.

Drug-likeness. Molecular weight, hydrogen-bond acceptors and donors, logP and molar refractivity were calculated with SwissADME [12] and Molinspiration [13] and assessed against Lipinski's rule of five [14].

2.8 Anti-tubercular activity (MABA)

Activity against *M. tuberculosis* H37Rv (ATCC 27294) was determined by the microplate Alamar Blue assay [15]. Sterile deionised water (200 μ L) was added to the outer wells of a 96-well plate to limit evaporation, and 100 μ L of Middlebrook 7H9 broth was added to the test wells. EEBP, dissolved in ethanol, was serially diluted directly in the plate to give final concentrations of 100 to 0.8 μ g/mL. Plates were sealed with parafilm and incubated at 37 °C for five days. Alamar Blue reagent and 10% Tween 80 (1:1; 25 μ L) were then added, and the plates were incubated for a further 24 h. A blue well indicated no bacterial growth and a pink well indicated growth; the MIC was the lowest concentration that prevented the colour change from blue to pink. Isoniazid, ethambutol, pyrazinamide, rifampicin and streptomycin were included as standard drugs.

3. Results and discussion

3.1 Organoleptic and morphological characters

The fresh leaves were green to dark green with a characteristic, slightly pleasant odour and slightly bitter taste. They were soft when fresh and somewhat leathery when dried, ovate to

lanceolate with an acute to acuminate apex, entire margin and reticulate venation. The ethanolic extract was likewise green to dark green. These features support identification of the leaf material and are recorded here as pharmacognostic reference characters.

3.2 Preliminary phytochemical screening

EEBP gave positive reactions for alkaloids, carbohydrates, glycosides, sterols, flavonoids, phenols, terpenoids and tannins (Table 1). These are qualitative tests of class, not of specific compounds, and several (for example the alkaloid and tannin reagents) can give false positives in crude extracts; they were therefore followed by chromatographic, spectrophotometric and GC-MS analyses

Class	Tests	Observation	Result
Alkaloids	Mayer's; Dragendorff's; Hager's	Creamy colour; orange-brown precipitate; yellow precipitate	+
Carbohydrates	Molisch's; Fehling's; Benedict's	Purple colour; reddish- brown precipitate; red precipitate	+
Glycosides	Borntrager's; ferric chloride	Pink colour; deep green to yellow colour	+
Sterols	Liebermann- Burchard	Brown ring at the junction; green upper layer	+
Flavonoids	Shinoda; alkali; lead acetate; acid	Red colour; reddish- brown precipitate; white precipitate; yellow colour	+
Phenols	Ferric chloride; lead acetate; ellagic acid	Bluish-black colour; precipitate; red colour	+
Terpenoids	Salkowski	Yellow ring turning red	+
Tannins	Phenazone; gelatin	Coloured precipitate; white precipitate	+

Table 1. Preliminary phytochemical screening of EEBP

3.3 Thin-layer chromatography

TLC supported the presence of phenolic, terpenoid and steroidal constituents (Table 2). The phenolic spot of EEBP (Rf 0.56, dark blue with ferric chloride) lay close to that of the gallic acid reference (Rf 0.60), indicating a phenolic compound of similar polarity but not confirming gallic acid itself. Single reddish-orange (Rf 0.50) and reddish-purple (Rf 0.52) spots with anisaldehyde-sulphuric acid are typical of terpenoids and steroids respectively. The terpenoid and steroid plates were run

without a reference spot for Rf comparison, so these assignments are class-level.

Constituent class	Mobile phase (v/v)	Detect ion	Spot colour	Rf (EEBP)
Phenolics	Acetic acid - chloroform (1:9)	10% ferric chloride	Dark blue	0.56 (gallic acid 0.60)
Terpenoids	Chloroform - methanol (9:1)	Anisaldehyde - sulphuric acid	Reddish orange	0.50
Steroids	Chloroform - methanol (95:5)	Anisaldehyde - sulphuric acid	Reddish purple	0.52

Table 2. TLC profile of EEBP

3.4 Quantitative estimation

All three calibration curves were linear over 10–50 µg/mL ($R^2 = 0.9729$ for ursolic acid, 0.9917 for cholesterol and 0.9952 for gallic acid; Figure 1). EEBP contained 22.57 mg UAE/g terpenoids, 24.62 mg CE/g sterols and 25.04 mg GAE/g phenolics (Table 3). Because these are colorimetric assays calibrated against a single reference compound, the values are equivalents that reflect the reactivity of the whole class with each reagent rather than absolute contents. They are, however, consistent with the GC-MS detection of triterpenoid, sterol and phenolic constituents (Section 3.5). The terpenoid curve had the lowest R^2 and the least favourable fit, so that estimate should be interpreted with the most caution.

Constituent	Standard	λ (nm)	Calibration equation	R^2	Content in EEBP (mg/g extract)
Terpenoids	Ursolic acid	538	$y = 0.0116x + 0.1862$	0.9729	22.57 (as UAE)
Sterols	Cholesterol	620	$y = 0.0099x + 0.0215$	0.9917	24.62 (as CE)
Phenols	Gallic acid	760	$y = 0.0233x - 0.0194$	0.9952	25.04 (as GAE)

UAE, ursolic acid equivalents; CE, cholesterol equivalents; GAE, gallic acid equivalents.

Table 3. Spectrophotometric estimation of phytoconstituents in EEBP

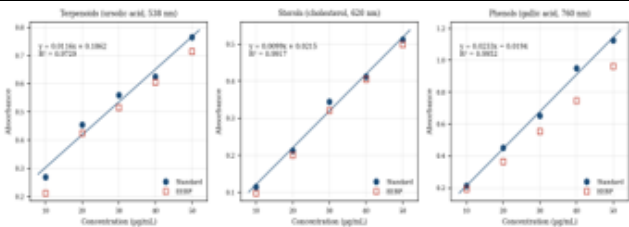


Figure 1. Calibration curves for terpenoids (ursolic acid), sterols (cholesterol) and phenols (gallic acid). Filled circles, standards; open squares, EEBP absorbance at the same nominal concentrations.

3.5 GC-MS profile

GC-MS analysis of EEBP resolved 60 peaks (Figure 2; complete list in Supplementary Table S1). Table 4 lists constituents at $\geq 1.0\%$ peak area together with the four minor constituents that were selected for docking. The profile contained carbohydrate-derived polyols and glycosides (methyl β -D-glucopyranoside, 4-C-methyl-myo-inositol), as expected for a polar ethanolic extract; phenolics (2-methoxy-4-vinylphenol, 2,6-dimethoxyphenol, 1,2,3-benzenetriol, 4-ethenyl-2,6-dimethoxyphenol); the flavone 3',4',5,7-tetrahydroxyflavone (luteolin); fatty-acid derivatives (n-hexadecanoic acid, cis-9-hexadecenal, 1-tetradecanol); sesquiterpenes (caryophyllene, cedrene); and the triterpenoid and sterol constituents epilupeol and γ -sitosterol. Epilupeol is the 3-epimer of lupeol, a triterpenoid already reported from B. prionitis [5], which is consistent with its detection here. Two cautions apply. First, compounds were assigned by NIST library matching alone, without authentic standards or retention indices, so identities are tentative. Second, diethyl phthalate is a common laboratory and plasticiser contaminant, and its presence cannot be attributed to the plant without a procedural blank.

Peak	Compound	RT (min)	Area (%)	Formula
5	Dihydroxyacetone	4.320	1.43	C ₃ H ₆ O ₃
20	Oxirane, (propoxymethyl)	8.062	1.71	C ₆ H ₁₂ O ₂
21	2-Propenoic acid, 2-methyl-, pentyl ester	8.321	9.06	C ₈ H ₁₄ O ₂
22	1-Butanol, 3-methyl acetate	8.617	1.55	C ₇ H ₁₄ O ₂
29	2-Methoxy-4-vinylphenol †	10.375	0.14	C ₉ H ₁₀ O ₂
34	1,2,3-Benzenetriol †	11.255	0.38	C ₆ H ₆ O ₃

37	2-Deoxy-D-galactose	12.033	1.38	C ₆ H ₁₂ O ₅
43	Diethyl phthalate †	13.669	0.74	C ₁₂ H ₁₄ O ₄
44	Methyl β-D-glucopyranoside	13.983	31.96	C ₇ H ₁₄ O ₆
45	myo-Inositol, 4-C-methyl-	13.646	21.91	C ₇ H ₁₄ O ₆
48	n-Hexadecanoic acid †	17.587	1.75	C ₁₆ H ₃₂ O ₂
50	cis-9-Hexadecenal	18.690	2.59	C ₁₆ H ₃₀ O
52	Epilupeol †	20.697	1.15	C ₃₀ H ₅₀ O
53	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.468	2.31	C ₁₉ H ₃₈ O ₄
54	1'H-Androst-16-enol[16,17-b]indol-3-ol, 1'-methyl-, (3β,5α)	22.835	1.00	C ₂₆ H ₃₃ NO
55	2,6-Dimethoxyphenol	22.987	3.67	C ₈ H ₁₀ O ₃
56	3',4',5,7-Tetrahydroxyflavone (luteolin)	23.437	2.24	C ₁₅ H ₁₀ O ₆
58	Decanedioic acid, bis(2-ethylhexyl) ester	25.073	3.39	C ₂₆ H ₅₀ O ₄
59	Dioncophylline A	26.123	4.83	C ₂₄ H ₂₉ NO ₃
60	γ-Sitosterol †	26.475	0.55	C ₂₉ H ₅₀ O

RT, retention time. † Selected for docking. Peak numbers follow the chromatogram in Figure 2.

Table 4. Major and docked constituents identified by GC-MS in EEBP

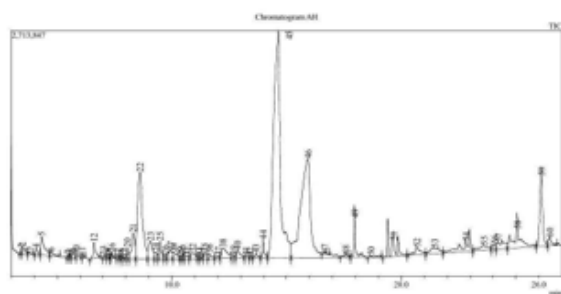


Figure 2. GC-MS total ion chromatogram of EEBP (peak numbers correspond to Table 4 and Supplementary Table S1).

3.6 Molecular docking and drug-likeness

All six ligands gave negative docking scores against InhA (Table 5). The lipophilic triterpenoid and sterol constituents epilupeol (−9.4 kcal/mol) and γ-sitosterol (−9.3 kcal/mol) scored highest, whereas the smaller phenolic and aliphatic compounds scored between −6.2 and −7.0 kcal/mol. In the 2D interaction maps (Figure 3), epilupeol formed a conventional hydrogen bond between its 3-hydroxyl group and Gly96 and a

π-σ contact with Phe149 and was further stabilized by an alkyl contact with Ala191 and van der Waals interactions with residues lining the pocket, including Ile16, Ile21, Met147, Tyr158 (a catalytic residue of InhA), Lys165 and Ile194. γ-Sitosterol was anchored by a hydrogen bond to Asp148, with alkyl and π-alkyl contacts with Ile16, Ile21, Phe41, Ile95, Met147 and Ala191.

The 0.1 kcal/mol difference between the two top ligands is well within the uncertainty of AutoDock scoring, so they should be regarded as equally ranked. Empirical scoring functions also tend to favour large, hydrophobic molecules, so part of the advantage of the triterpenoid and sterol ligands over the small phenolics probably reflects size and surface area rather than specific recognition. Docking scores are predictions and do not establish enzyme inhibition.

Ligand	Docking score (kcal/mol)	MW (g/mol)	HBA	HBD	LogP	MR	Lipinski violations
Epilupeol	−9.4	426.70	1	1	9.90	130.65	1 (LogP > 5)
γ-Sitosterol	−9.3	414.72	1	1	9.30	128.22	1 (LogP > 5)
2-Methoxy-4-vinylphenol	−7.0	150.17	2	1	2.40	44.75	0
Diethyl phthalate	−6.9	222.24	4	0	2.50	58.36	0
1,2,3-Benzenetriol	−6.3	126.11	3	3	0.50	31.44	0
n-Hexadecanoic acid	−6.2	256.43	1	1	6.40	77.95	1 (LogP > 5)

Table 5. Docking scores against InhA and physicochemical properties of the docked ligands

MW, molecular weight; HBA, hydrogen-bond acceptors; HBD, hydrogen-bond donors; MR, molar refractivity (cm³/mol). Lipinski limits: MW < 500, HBA < 10, HBD < 5, LogP < 5; MR < 150 is an extended criterion.

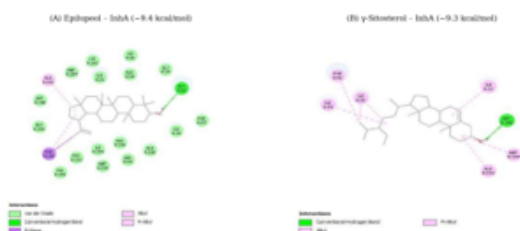


Figure 3. 2D interaction diagrams of (A) epilupeol and (B) γ -sitosterol docked in the InhA binding site.

Against Lipinski's criteria [14], diethyl phthalate, 1,2,3-benzenetriol and 2-methoxy-4-vinylphenol had no violations. Epilupeol, γ -sitosterol and n-hexadecanoic acid each exceeded the logP limit (calculated logP 9.90, 9.30 and 6.40 respectively), and Molinspiration miLogP values for the two triterpenoid and sterol compounds were also above 8. The strongest-binding ligands are therefore highly lipophilic, and poor aqueous solubility would be expected to limit oral bioavailability. They are better regarded as lead scaffolds, or as contributors to the activity of the extract, than as drug-like candidates without formulation or derivatization.

3.7 Anti-tubercular activity

EEBP prevented the blue-to-pink colour change at 100, 50 and 25 $\mu\text{g/mL}$ and permitted growth at 12.5 $\mu\text{g/mL}$ and below (Figure 4B), giving an MIC of 25 $\mu\text{g/mL}$ against *M. tuberculosis* H37Rv (Table 6). Under the same assay the standard drugs gave MICs between 0.8 and 3.125 $\mu\text{g/mL}$ (Figure 4A). EEBP was therefore about eight-fold less potent than pyrazinamide and roughly 16- to 31-fold less potent than isoniazid, ethambutol, rifampicin and streptomycin. This is expected when an unfractionated extract is compared with pure drugs; even so, an MIC of 25 $\mu\text{g/mL}$ for a crude leaf extract indicates appreciable antimycobacterial activity and supports bioassay-guided fractionation. Triterpenoids and sterols such as those detected by GC-MS belong to compound classes previously linked to antimycobacterial activity in other plants [4,16], but the present data do not identify which constituents are responsible.

Test material	MIC ($\mu\text{g/mL}$)	EEBP MIC relative to material
EEBP	25	—
Isoniazid	1.6	$\approx 16\times$
Ethambutol	1.6	$\approx 16\times$
Pyrazinamide	3.125	$8\times$
Rifampicin	0.8	$\approx 31\times$
Streptomycin	0.8	$\approx 31\times$

EEBP tested at 100, 50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 $\mu\text{g/mL}$: no growth at 100, 50 and 25 $\mu\text{g/mL}$; growth at all lower concentrations.

Table 6. MIC of EEBP and standard anti-tubercular drugs against *M. tuberculosis* H37Rv (MABA)

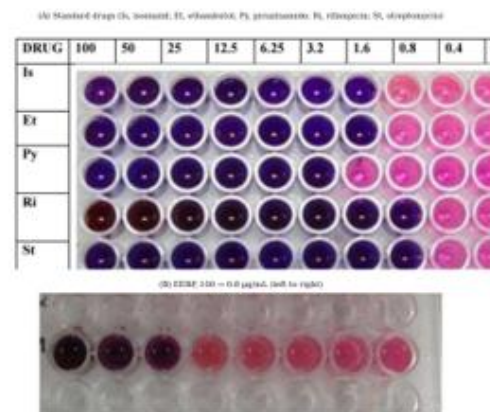


Figure 4. MABA plates. (A) Standard anti-tubercular drugs; (B) EEBP, 100 to 0.8 $\mu\text{g/mL}$. Blue/purple wells, no growth; pink wells, growth.

3.8 Interpretation and limitations

The computational and microbiological results are complementary but not directly linked. MABA reports whole-cell growth inhibition by a crude, multi-component extract, whereas docking addresses a single putative target for individual compounds. The data do not show that InhA inhibition underlies the observed activity, or that epilupeol or γ -sitosterol are responsible for it. Further limitations are that the compounds were not isolated or confirmed against authentic standards, that cytotoxicity and selectivity were not assessed, and that the activity of a single extract was tested against a single reference strain.

4. Conclusions

The ethanolic leaf extract of *B. prionitis* contains phenolics, terpenoids, sterols and other metabolites (60 GC-MS peaks) and inhibited *M. tuberculosis* H37Rv in the MABA with an MIC of 25 $\mu\text{g/mL}$, about eight-fold higher than pyrazinamide

under the same conditions. Docking suggested that epilupicol and γ -sitosterol may bind InhA (−9.4 and −9.3 kcal/mol), although their high lipophilicity limits Lipinski compliance. These findings justify bioassay-guided fractionation, isolation of the triterpenoid and sterol constituents, InhA enzyme-inhibition and cytotoxicity/selectivity assays, and subsequent in-vivo evaluation.

Author Contributions: Murugesan S: Conceptualization, methodology, investigation, extraction, data collection and analysis, characterization, spectroscopic data analysis and interpretation, In silico analysis, biological data analysis and interpretation. Poovizhi M: writing—original draft. Sivasubramanian P: Supervision, project administration, validation, critical review. Kavisri R, Sergin P: editing of the manuscript. All authors contributed to the interpretation of the results, reviewed the manuscript critically, and approved the final version.

Conflicts of Interest: The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

No.	Compound	RT (min)	Area (%)	Formula
1	2H-Pyran-2-one	3.425	0.10	C ₅ H ₄ O ₂
2	Glyceraldehyde	3.482	0.41	C ₃ H ₆ O ₃
3	2-Furanmethanol	3.759	0.12	C ₅ H ₆ O ₂
4	Propanoic acid, 2-oxo-, methyl ester	4.092	0.29	C ₄ H ₆ O ₃
5	Dihydroxyacetone	4.320	1.43	C ₃ H ₆ O ₃
6	1,2-Cyclopentanedione	4.729	0.29	C ₅ H ₆ O ₂
7	2(5H)-Furanone, 3-methyl	5.475	0.07	C ₅ H ₆ O ₂
8	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	5.552	0.11	C ₆ H ₈ O ₄
9	Glycerin	5.748	0.24	C ₃ H ₈ O ₃
10	Butanoic acid, 3-hydroxy	5.840	0.21	C ₄ H ₈ O ₃
11	1-Hexanol	6.091	0.06	C ₆ H ₁₄ O
12	4-Chloro-3-methylbut-2-en-1-ol	6.604	0.85	C ₅ H ₉ ClO
13	1,3,5-Triazine-2,4,6-triamine	7.030	0.09	C ₃ H ₆ N ₆
14	1-Butanol, 2-nitro	7.180	0.05	C ₄ H ₉ NO ₃
15	Cyclopropyl carbinol	7.300	0.01	C ₄ H ₈ O
16	2-Buten-1-ol, (Z)-, TMS derivative	7.386	0.03	C ₇ H ₆ OSi
17	(2-Piperidin-3-ylethyl)amine	7.647	0.01	C ₇ H ₁₆ N ₂
18	Pentanoic acid, 4-oxo	7.750	0.04	C ₅ H ₈ O ₃
19	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	7.879	0.33	C ₆ H ₈ O ₄
20	Oxirane, (propoxymethyl)	8.062	1.71	C ₆ H ₁₂ O ₂
21	2-Propenoic acid, 2-methyl-, pentyl ester	8.321	9.06	C ₈ H ₁₄ O ₂
22	1-Butanol, 3-methyl acetate	8.617	1.55	C ₇ H ₁₄ O ₂
23	5-Hydroxymethylfurfural	9.066	0.38	C ₆ H ₆ O ₃
24	1,2,3-Propanetriol, 1-acetate	9.291	0.78	C ₅ H ₈ O ₄
25	Acetic acid, hexyl ester	9.487	0.22	C ₈ H ₁₆ O ₂
26	Diethoxymethyl acetate	9.705	0.66	C ₇ H ₁₄ O ₄
27	1,3-Propanediol, 2-hydroxymethyl-2-methyl	9.948	0.58	C ₅ H ₁₂ O ₃
28	Dimethyl-cyano-phosphine	10.084	0.11	C ₃ H ₆ NP
29	2-Methoxy-4-vinylphenol	10.375	0.14	C ₉ H ₁₀ O ₂
30	2,5-Monomethylene-1-rhamnitol	10.487	0.25	C ₇ H ₁₄ O ₅
31	Isosorbide dinitrate	10.685	0.56	C ₆ H ₈ N ₂ O ₈
32	3-Ethylthio-1-propene	10.906	0.09	C ₅ H ₁₀ S
33	Benzenemethanol, 4-(1,1-dimethylethyl)	11.175	0.07	C ₁₁ H ₁₆ O

No.	Compound	RT (min)	Area (%)	Formula
34	1,2,3-Benzenetriol	11.255	0.38	C ₆ H ₆ O ₃
35	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethan-1-one	11.455	0.34	C ₁₃ H ₁₈ O ₂
36	Caryophyllene	11.634	0.17	C ₁₅ H ₂₄
37	2-Deoxy-D-galactose	12.033	1.38	C ₆ H ₁₂ O ₅
38	Rishitin	12.230	0.23	C ₁₄ H ₂₂ O ₂
39	β-D-Glucopyranose, 1,6-anhydro	12.695	0.69	C ₆ H ₁₀ O ₅
40	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	12.862	0.22	C ₁₅ H ₂₄
41	Cedrene	13.230	0.08	C ₁₅ H ₂₈
42	Phenol, 4-ethenyl-2,6-dimethoxy	13.415	0.31	C ₁₀ H ₁₂ O ₃
43	Diethyl phthalate	13.669	0.74	C ₁₂ H ₁₄ O ₄
44	Methyl β-D-glucopyranoside	13.983	31.96	C ₇ H ₁₄ O ₆
45	myo-Inositol, 4-C-methyl-	13.646	21.91	C ₇ H ₁₄ O ₆
46	1-Tetradecanol	15.939	0.10	C ₁₄ H ₃₀ O
47	Propanamide, N-(4-ethoxyphenyl)	16.675	0.19	C ₁₁ H ₁₅ NO ₂
48	n-Hexadecanoic acid	17.587	1.75	C ₁₆ H ₃₂ O ₂
49	Phenol, o-(benzylthio)	17.982	0.15	C ₁₃ H ₁₂ OS
50	cis-9-Hexadecenal	18.690	2.59	C ₁₆ H ₃₀ O
51	4,6-O-Ethylidene-α-D-glucose	19.670	0.97	C ₈ H ₁₄ O ₆
52	Epilupeol	20.697	1.15	C ₃₀ H ₅₀ O
53	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.468	2.31	C ₁₉ H ₃₈ O ₄
54	1'H-Androst-16-eno[16,17-b]indol-3-ol, 1'-methyl-, (3β,5α)	22.835	1.00	C ₂₆ H ₃₃ NO
55	2,6-Dimethoxyphenol	22.987	3.67	C ₈ H ₁₀ O ₃
56	3',4',5,7-Tetrahydroxyflavone	23.437	2.24	C ₁₅ H ₁₀ O ₆
57	Phenol, 3-pentadecyl	24.893	0.37	C ₂₁ H ₃₆ O
58	Decanedioic acid, bis(2-ethylhexyl) ester	25.073	3.39	C ₂₆ H ₅₀ O ₄
59	Dioncophylline A	26.123	4.83	C ₂₄ H ₂₉ NO ₃
60	γ-Sitosterol	26.475	0.55	C ₂₉ H ₅₀ O

Supplementary Table S1. Compounds detected by GC-MS in the ethanolic leaf extract of *B. prionitis* (60 peaks)