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Flavonoids from *Alpinia officinarum* as Potential Anti-Tubercular Agents Using Molecular Docking Studies

VARSHA S. HONMORE[1,*], ARUN D. NATU[1], VIJAY M. KHEDKAR[2], DHIMAN SARKAR[3] and SUPADA R. ROJATKAR[4]

[1]Post Graduate and Research Center, Department of Chemistry, MES Abasaheb Garware College, Pune-411004, India
[2]Department of Pharmaceutical Chemistry, School of Pharmacy, Vishwakarma University, Pune-411048, India
[3]Combichem-Bioresource Center, Organic Chemistry Division, CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pashan, Pune-411008, India
[4]R&D Centre in Pharmaceutical Sciences and Applied Chemistry, Poona College of Pharmacy Campus, Bharati Vidyapeeth Deemed University, Erandwane, Pune-411038, India

*Corresponding author: Fax: +91 20 25438165; E-mail: vshonmore@yahoo.com; vhonmore@gmail.com

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Bioguided isolation yielded three flavonoids galangin (**1**), kaempferide (**2**) and kaempferol (**3**) from methanol extract of *Alpinia officinarum* Hance. Galangin exhibited highest antitubercular activity, with MIC of < 4.0 µg/mL against *M. bovis* BCG (*in vitro*) and *M. tuberculosis* (*ex vivo*), than kaempferide. *In vitro* and *ex vivo* macrophage infection model assay revealed the inhibition of both active and dormant stage for *M. tuberculosis* H37Ra and *M. bovis* BCG on exposure to galangin. Molecular docking studies into the active site of DprE1 enzyme helped to understand the ligand-protein interactions and establish a structural basis for inhibition of *M. tuberculosis*. These flavonoids indicated their non-specificity towards *M. tuberculosis* by testing against Gram-positive and Gram-negative bacteria and also least cytotoxic up to 100 µg/mL on three human cancer cell lines THP-1, PANC-1 and A549, respectively. So these flavonoids are inhibitors against *M. tuberculosis* that can be explored further as potential antitubercular drugs.

Keywords: *Alpinia officinarum*, Zingebareace, Antimycobacterial agent, Galangin, Kaempferide, Molecular docking.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is a threat to human health [1]. These Gram-positive, acid-fast bacteria lead to the problems as follows. First, human immunodeficiency virus (HIV), which is widely distributed, weakens the immune system, resulting in the spread of tuberculosis. Second, the origin of multidrug-resistant and extremely drug-resistant strains of MTB has made the treatment considerably difficult. Finally, a minimum of six months are require for drug therapy and many antitubercular drugs have severe side effects [2]. Thus, WHO declared tuberculosis to be global emergency because of its latent haven in approximately one-third of global population. Therefore, in search of alternative antitubercular agents, we concentrated our efforts towards screening of *Alpinia officinarum* Hance from zingebaraceae family, against MTB and isolating the marker compounds.

The rhizomes are ethnomedically most valued part of *A. officinarum* Hance [3]. The broad spectrum of biological activities of this plant is mainly due to presence of diarylhepatanoids [4,5], flavonoids [6,7] and phenylpropanoids [8]. The anti-tubercular activity of diarylheptanoids from *A. officinarum* has been reported [9].

With this background, to elucidate the possible mechanism underlying their antitubercular activity, molecular docking exercise was carried out to visualize the binding mode at the molecular level. Here, we have presented results of bio-guided isolation, purification of flavonoids from *A. officinarum*, their anti-mycobacterial and antimicrobial activity with cytotoxicity.

EXPERIMENTAL

Plant material: Dried rhizomes of *A. officinarum* were purchased from Kerala state, India. These were authenticated by the Department of Botany, Agharkar Research Institute,

Pune and voucher specimen (R-138) of plant material is maintained in the laboratory.

Extraction and bio-guided isolation of marker compounds: A rhizome (150 g) was grounded and then extracted through maceration at room temperature (48 h × 3) with methanol (500 mL × 3). The obtained viscous extracts were filtered and then concentrated using a rotary evaporator at reduced pressure of 40 ºC, thereby obtaining a crude methanol extract (11.7 g). This crude extract was screened and determined as effective against MTB. Therefore, this extract was subjected to fractionation by using different solvents after further purification.

The methanol extract (7.0 g) was redissolved in methanol: water (80:20, 100 mL) and partitioned with *n*-hexane followed by ethyl acetate. These fractions along with aqeous methanol layer were concentrated in vacuum and screened against MTB. The results of fractionation and antitubercular screening are collectively represented in Table-1. From these results, it was evident that the ethyl acetate fraction exhibited prominent inhibitory action against MTB endorsing further purification leading to marker compounds.

TABLE-1
ANTITUBERCULAR ACTIVITY OF EXTRACTS, FRACTIONS AND COMPOUNDS FROM *Alpinia officinarum* HANCE USED FOR PRIMARY SCREENING

Extract/compound	Fraction	Activity % inhibition
Crude extract	Methanol	85.20 (at 25 µg/mL)
Sub-fractions by solvent extraction	*n*-Hexane	59.00 (at 100 µg/mL)
	Ethyl acetate	83.90 (at 12.5 µg/mL)
	Aq. methanol	27.00 (at 100 µg/mL)
Ethyl acetate	AO-4	98.63 (at 12.5 µg/mL)
	AO-5	95.01 (at 6.25 µg/mL)
1	Galangin	91.88 (at 30 µg/mL)
2	Kaempferide	89.77 (at 30 µg/mL)
3	Kaempferol	48.65 (at 30 µg/mL)

A fraction of ethyl acetate (1.4 g) was analyzed through column chromatography on silica gel (100-200 mesh) with the mobile phase of a hexane-ethyl acetate gradient (0-100%). AO-1 to AO-7 fractions were acquired. Among the seven fractions, AO-4 (0.025 g) and AO-5 (0.134 g) were further purified. After purification through crystallization, fraction AO-4 provided compound **1** (10 mg). After purification through repeated chromatography and preparative TLC, whereas fraction AO-5 afforded compounds **2** (7 mg) and **3** (5 mg). The antitubercular activity of compounds (**1–3**) was also investigated. Only compounds **1** and **2** exhibited good results.

Antitubercular activity: The standard cultures of *M. bovis* BCG (ATCC 35743) and MTB H37Ra (ATCC 25177), hereafter represented as MTB, were purchased from the American Type Culture Collection (Manassas, USA). *M. bovis* BCG and MTB were grown in 50 mM sodium nitrate containing Dubos media (Difco, Detroit, USA) and specific *Mycobacterium phlei* media [10,11], respectively. These media were stored as glycerol stocks at −70 ºC. Before inoculation for the experiment, glycerol stock (50 µL) was pre-inoculated in a corresponding medium for obtaining mycobacteria that was metabolically active. In each experiment, under aerobic conditions, the culture was grown to log phase [optical density at 595 nm (OD_{595}) = 1] at 37 ºC and 150 rpm. Because in the culture medium, *mycobacteria* grew in aggregated clumps, these cultures were sonicated for 2 min by employing a water-bath sonicator (Ultrasonic, Freeport, USA) to afford viable dispersed cells. This process was incorporated for reproducibly inoculating mycobacterial bacilli in a fresh medium for conducting experiments.

Primary screening: The inhibition activity of compounds afforded from the rhizome extract against dormant (12 days incubation) and active (8 days incubation) mycobacteria was screened at the concentrations of 10, 30 and 100 µg/mL. Their activity against MTB was estimated by employing the absorbance reading for XTT reduction menadione assay (XRMA) at 470 nm according to a protocol described in the literature [12]. The nitrate reductase (NR) assay was studied to determine *M. bovis* BCG inhibition [10,11]. For the NR assay, absorbance was determined at 540 nm. The following formula can be used to calculate percentage inhibition:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{CMPD}}{\text{Control} - \text{Blank}} \times 100$$

where control is the activity of mycobacteria without compounds, CMPD is the activity of mycobacteria with compounds acquired from rhizome and blank is the activity of the culture medium without mycobacteria.

The experiments were performed in triplicates. The quantitative values were expressed as the mean ± standard deviation (S.D.).

Determination of 50% inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC): Depending on the primary screening results, the MIC and IC_{50} of active compounds against mycobacteria were evaluated by employing the dose-response assay for the concentration of 0.03-30 µg/mL. Origin Pro software (Origin Lab Corp., Northampton, USA) was used to plot the dose-response curve. IC_{50} and MIC were considered the lowest concentrations of compounds showing growth inhibition of ≥ 90% and 50%, respectively, in relation to that of the growth control without compounds. Rifampicin, the standard antitubercular drug, was considered the positive control and purchased from Sigma (≥ 97% HPLC purity). All the experiments were performed in triplicates.

***In vitro* assay:** The *In vitro* activity of the compounds against *M. bovis* BCG and MTB at dormant (12 days) and active (8 days) stages was analyzed using NR and XRMA assay, respectively.

***Ex vivo* infection model assay:** Human acute monocytic cell lines THP-1 were obtained from the National Centre for Cell Science, (Pune, India). In RPMI 1640 (HiMedia, Mumbai, India) medium with 10% foetal bovine serum (Gibco, India), 1% non-essential amino acids (HiMedia), 1 mM sodium pyruvate (HiMedia) and 1% glutamine (HiMedia), the THP-1 cells were cultured through incubation at 37 ºC in the atmosphere of 95% relative humidity and 5% CO_2 in a CO_2 incubator (Thermo Fisher Scientific, USA). For the infection model analysis, in 96-well microtiter plates (Tarsons, India), THP-1 (3×10^5 cells/mL were passaged in a complete RPMI medium with 100 nM/mL phorbol myristate acetate (Sigma Chemical Co.) and was plated for differentiation to macrophages. These cells were

infected with MTB (OD_{595} = 1) after 24 h at the multiplicity of infection of 100 for 12 h. After thorough washing with phosphate buffered saline (PBS) (pH 7.2), a fresh minimum essential medium (HiMedia) with 50 mM sodium nitrate (HiMedia) was added to the plates. Then, the infected cells were treated with compounds at various concentrations. The plates were further incubated for 12 (dormant) and 8 (active) days after the estimation of compound activities through the NR assay [13]. The experiments were performed in triplicates. MIC and IC_{50} were calculated using the dose-response curves plotted with Origin 8 software (Origin Lab Corp).

Antimicrobial activity: The specificity of compounds was determined by testing their concentrations of 0.1, 0.3, 1, 3, 10, 30, and 100 µg/mL against bacteria. The bacterial strains comprised two Gram-positive bacteria, namely *Bacillus subtilis* (ATCC23857) and *Staphylococcus aureus* (ATCC29213), and two Gram-negative bacteria, namely *Escherichia coli* (ATCC-25292) and *Pseudomonas fluorescens* (ATCC13525). For the antimicrobial studies [14-16], OD-adjusted (OD_{620} = 1) cultures were inoculated in the LB broth (1% v/v). Subsequently, in a 96-well microtitre plate, the compounds (2.5 µL) and culture (247.5 µL) were dispensed and were incubated at 37 ºC for 18 h before absorbance measurement at 620 nm. Ampicillin, purchased from Sigma (≥ 96% purity), was employed as the positive control. For sterility control and growth, wells were also included. IC_{50} and MIC were calculated using the dose-response curve.

Cytotoxicity assay: The *In vitro* potential impacts of compounds (**1-3**) on cell viability were studied through the reduction of tetrazolium (MTT) dye [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich, USA), a widely adopted method for measuring cellular proliferation, as reported in the literature [17-20]. The influences of flavonoids on cell growth were studied with a panel of human tumour cells, such as acute monocytic leukaemia cell line (THP-1), lung adenocarcinoma (A549), and pancreatic adenocarcinoma (Panc-1), acquired from the European Collection of Cell Cultures (Salisbury, UK). The following formula can be used to calculate percentage cytotoxicity:

$$\text{Cytotoxicity (\%)} = \frac{\text{Control} - \text{CMPD}}{\text{Control} - \text{Blank}} \times 100$$

where control is the cell growth in the medium without compounds, CMPD is the cell growth with compounds obtained from rhizome, and blank is the culture medium without cells. Paclitaxel (96% purity), purchased from Sigma, was utilised as the positive control. The experiments were performed in triplicates. The quantitative values were expressed as the mean ± S.D.

Molecular Docking: The promising levels of antitubercular activity of isolated flavonoids acquired in cell based assays led us to conduct molecular docking to estimate the interaction mode and binding affinity of these compounds in the active sites of the MTB DprE1 enzyme. The grid-based ligand docking with energetics (GLIDE) [21,22] module combined with the Schrödinger molecular modelling package (Schrödinger, USA 2016) was employed for this study. The oxidase participating in arabinogalactan biosyntheses (decaprenylphosphoryl-D-ribose oxidase (DprE1)) catalyzed the FAD-dependent oxidation of decaprenylphosphoryl-β-D-ribose(DPR) into decaprenylphosphoryl-2-keto-D-erythro-pento furanose (DPX). Then, the NADH-dependent enzyme DprE2 converted DPX into decaprenyl phosphoryl-β-D-arabinose (DPA), the central precursor for the mycobacterial cell-wall cores and the only known donor of D-arabinofuranosyl residues for arabinogalactan syntheses [23,24]. Therefore, DprE1 is necessary for the viability of MTB, making it a promising target for the strategy of antimycobacterial drug design. Due to its inhibition through multiple pharmacophores that span diverse chemical spaces, DprE1 is considered the promiscuous target [25,26]. This fact encouraged DprE1 selection for evaluating the binding potential of isolated compounds for the wall target of crucial mycobacterial cells.

In complex with an inhibitor, the MTB DprE1(decaprenyl phosphoryl-β-D-ribose-2′-epimerase) crystal structure (PDB code:4FDO) was acquired from the Protein Data Bank. This structure refined by employing protein preparation wizard utilized for docking. By removing all crystallographically found water molecules (because none of them were conserved during interactions with ligands or proteins), identifying overlaps, assigning bond orders, creating disulphide bonds and creating zero order bonds to metals, the crystal structure was preprocessed. Missing side chain/hydrogen atoms corresponding to pH 7 were added, which assigned appropriate ionisation states for basic and acidic amino acids. Appropriate protonation states and charges were assigned to the acquired structure. Finally, using the optimized potentials for liquid simulation (OPLS-2005) force-field with root mean square deviation (RMSD) of 0.30 Å, energy minimization was performed to obtain steric clashes, resulting from hydrogen atom addition, among residues.

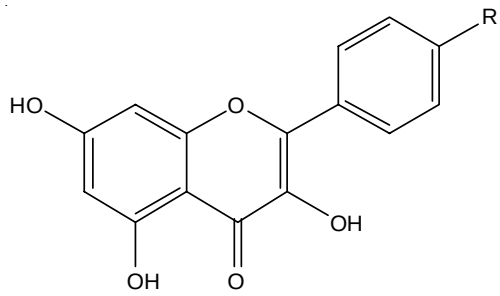
The active sites of an enzyme were defined with the *Receptor Grid Generation* panel. This panel produces two cubical boxes, a small binding box and a large enclosing box, with a common centroid for calculations. For the complex, the inhibitor location was known; thus, active sites were defined using a cubic grid 12 × 12 × 12 Å dimension box placed on the native ligand centroid in the crystal complex. These sites were adequately large to study the larger regions of enzyme structures.

Using a *builder* panel in Maestro, the 3D structures of these compounds were sketched. Subsequently, these structures were optimised using the *Ligprep* module used to add hydrogen atoms by adjusting realistic bond angles and lengths and to correct ionization states, chiralities, tautomers, ring conformations and stereochemistries. A partial atomic charge was assigned to a structure by using the OPLS-2005 force field. The charge was then optimized through energy minimisation until 0.01 kcal/ mol/Å gradient was attained. With this setup, automated docking was performed to gauge the binding affinity of these compounds in DprE1 macromolecules. The Glide algorithm uses an approach of funnel-type filtering, in which systematic search can be performed to identify a favourable interaction between macromolecules and ligand molecules, where each ligand pose travels through several hierarchical filters, which determine the interaction of the ligands with

receptors. Finally, to measure the binding affinity of the ligands and rank the resulting docked poses, each docking solution can be scored using Schrödinger's proprietary glide score multi-ligand scoring function. Using Maestro's pose viewer utility, the output was investigated to acquire the key interaction elements for enzymes.

RESULTS AND DISCUSSION

The isolated compounds (**1-3**) were further confirmed as galangin (**1**) [27], kaempferide (**2**) [28] and kaempferol (**3**) (Fig. 1) [29] on the basis of spectral analysis (IR, ^{1}H & ^{13}C NMR and MS). The purity of compound **1** was confirmed by HPLC (97.4%, Fig. 2) as this compound was isolated in a major quantity while compounds (**2** and **3**) by ^{1}H & ^{13}C NMR spectra which are in a minor quantity.



1. R = H
2. R = OCH_3
3. R = OH

Fig. 1. Chemical representation of compounds **1-3**

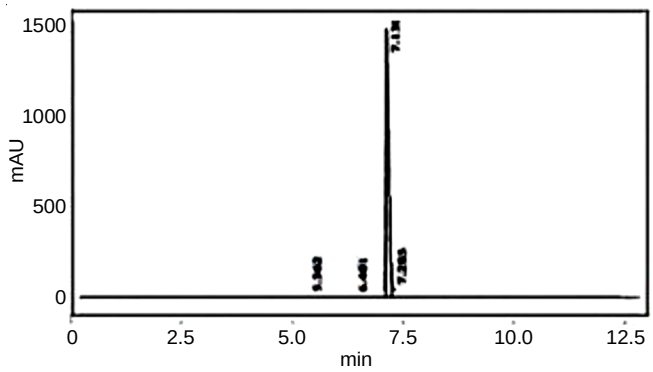


Fig. 2. HPLC of compound **1**

In present study, the compounds were evaluated for their anti-tubercular activity against non-virulent strain of MTB (MTB H37Ra; ATCC 25177) in liquid medium. It may be noted here that the efficiency of inhibiting MTB by crude extract of rhizomes observed in *Mycobacterium tuberculosis* H37Ra was less as compared to those observed with isolated flavonoids.

In preliminary screening, all three compounds were tested against active and dormant MTB at concentrations 10, 30 and 100 µg/mL using XRMA anti-tubercular screening protocol. Table-1 represents the 90% inhibition obtained at different concentrations of compounds. Rhizomes extracts and compounds (**1** and **2**) exhibited profound mycobactericidal potency, inhibiting > 90% of mycobacterial growth. On exposure to other extracts, 50-85% inhibition was observed except aq. methanol fraction, which showed up to 27% inhibition against mycobacteria.

These compounds were further tested to determine their MIC and IC_{50} against mycobacteria under both *in vitro* and within THP-1 host macrophages (Table-2). *In vitro* studies against *M. bovis* BCG and *ex vivo* studies against MTB revealed the strong anti-tubercular activity of compounds (**1** and **2**). Compound **1** was found to be highly effective to inhibit both active and dormant *M. bovis* BCG (*in vitro*) and mycobacteria (*ex vivo*) with MIC ranged from 0.97 to 3.10 µg/mL and IC_{50} 0.039 to 1.48 µg/mL. However, by *in vitro* the activity is not profound against mycobacteria and a still higher concentration compound (MIC/IC_{50} = 28.58/1.94 µg/mL) is required for complete mycobacterial inhibition. Compound **2** exhibited least activity where MIC was >100 µg/mL and IC_{50} was > 5 µg/mL. But, compound **2** showed much better mycobactericidal activity under *ex vivo* condition with MIC ranged from 0.94 to 2.38 µg/mL and IC_{50} 0.018 to 0.087 µg/mL. MIC of dormant stage mycobacteria was observed to be greater than its active stage. In view of this, the overall anti-tubercular activity exhibited by compounds (**1** and **2**) is significant; although they possess lower potencies as compared to that of standard drug, rifampicin.

In order to check the specificity of compounds (**1-3**) for *mycobacteria*, these compounds were tested against Gram-positive and Gram-negative bacteria to determine MIC and IC_{50} (Table-3). This indicates that compound **2** isolated from extract, have greater specificity towards *mycobacteria* as compared to that of compound **1**.

TABLE-2
ANTITUBERCULAR ACTIVITY OF COMPOUNDS BY *in vitro* AND *ex vivo* ASSAY

Compound	*in vitro* activity								*ex vivo* activity against *M. tuberculosis* H37Ra			
	M. tuberculosis H37Ra				*M. bovis* BCG							
	Dormant		Active		Dormant		Active		Dormant		Active	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
1	1.63	28.58	1.94	32.87	1.48	3.10	1.44	2.50	0.039	0.97	0.16	2.47
2	5.24	>100	12.05	>100	7.84	27.31	9.86	83.93	0.018	0.94	0.087	2.38
Rifampicin[a]	0.0014	0.043	0.0018	0.048	0.0014	0.073	0.0018	0.078	0.0018	0.048	0.0021	0.051

Compounds (**1** and **2**); BCG, *Mycobacterium bovis* BCG (ATCC 35743); MTB, *Mycobacterium tuberculosis* H37Ra (ATCC 25177); IC_{50} and MIC, lowest concentration of compounds exhibiting growth inhibition of 50% and ≥ 90%, respectively, relative to the growth control without compounds. All values are in µg/mL. The experiment was performed in triplicate and mean values were plotted in a dose–response curve to obtain IC_{50} and MIC values of compounds.
[a]The standard antitubercular drugs rifampicin was used as a positive control.

TABLE-3
ANTIBACTERIAL ACTIVITY OF FLAVONOIDS (**1-3**) AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Compound	*Staphylococcus aureus*		*Bacillus subtilis*		*Pseudomonas fluorescens*		*Escherichia coli*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
1	12.40	20.90	7.80	39.71	24.22	43.44	35.43	45.70
2	>100	>100	>100	>100	68.57	>100	73.75	88.43
3	>100	>100	>100	>100	>100	>100	>100	>100
Ampicillin	0.12	1.0	5.89	10.32	2.83	4.36	0.41	1.46
Rifampicin	16	>30	0.25	1.35	0.012	0.49	0.67	1.62

Flavonoids; IC_{50} and MIC, lowest concentration of compounds (**1-3**) exhibiting growth inhibition of 50% and ≥ 90%, respectively, relative to the growth control without compounds. All values are in µg /mL. The experiment was performed in triplicate and mean values were plotted in a dose-response curve to obtain IC_{50} and MIC values of compounds.

The antimicrobial activities of compounds **1-3** against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens* were tested. For compounds **2** and **3**, both Gram-positive and Gram-negative bacteria exhibited a high resistance with MIC > 100 µg/mL. The MIC of compound **1** against these bacteria was 20-46 µg/mL (Table-3).

The rhizome extract is considered non-toxic and safe. Thus, the anti-proliferative activity of compounds **1** and **2** was tested against human cancer cell lines. Up to 100 µg/mL THP-1, A549, and PANC-1 cell lines, compounds **1** and **2** exhibited least cytotoxicity (Table-4).

Fig. 3 presents percentage cytotoxicity acquired through MTT cell proliferation against the cell lines for 100 µg/mL compounds with different origin. For *mycobacteria*, the tested concentration was nearly 10 times higher than the *ex vivo* observed MIC. At such a high compound concentration after 48 h treatment, 48% inhibition was observed, which suggested compounds' biocompatible nature.

To propose the structural hypotheses for how ligands inhibit the target and predict the predominant binding modes of the ligands within proteins of known 3D structures, molecular docking is the key approach to computer assisted drug design. Inhibition is critical in lead optimization, thus, to acquire insights into inhibition patterns and rationalize the antitubercular results, interactions between the binding pocket of mycobacterial DprE1 and isolated compounds **1** and **2** were explored and depicted through molecular docking. Visual compound analyses for the docked poses with the minimum energy revealed that these compounds could fit into the DprE1 binding pocket at co-ordinates near those of native ligands in the crystal structure; thus they, make close contact with surrounding residues. Table-5 presents the values of the binding energy, docking scores and

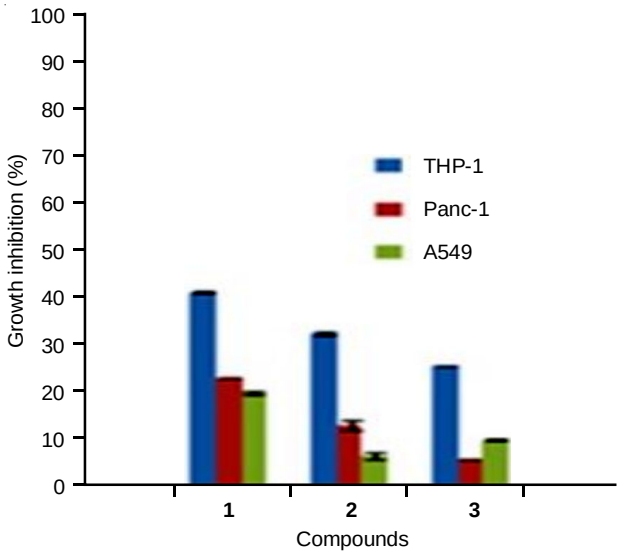


Fig. 3. Percentage cytotoxicity of compounds at 100 µg/mL against human cell lines

non-covalent interactions for these molecules. A detailed analysis of per-residue interactions between the protein and compound (**2**, kaempferide) was also performed and is elucidated, which one can speculate regarding the binding patterns in the cavity while the results for compound (1, galangin) are depicted in (Fig. 4).

The binding mode of compound **2** into the active site of DprE1 is shown in Fig. 5. Though the compound is found to be engaged in multiple interactions with the residues in the active site, however for the sake of visibility and clarity only selected interacting residue are shown. Quantitative analysis of the docked complex showed a strong binding affinity

TABLE-4
CYTOTOXIC ACTIVITY OF COMPOUNDS (**1-3**) FOR 3 HUMAN CANCER CELL LINES AFTER 48 h OF EXPOSURE[a]

Compound	Cytotoxic profile against human cancer cell lines with SD values					
	THP-1		Panc-1		A549	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
1[b]	>100	>100	>100	>100	>100	>100
2[b]	>100	>100	>100	>100	>100	>100
3[b]	>100	>100	>100	>100	>100	>100
[c]Paclitaxel	0.1374 ± 0.53	5.8140 ± 0.02	0.1279 ± 0.96	5.7150 ± 0.19	0.0035 ± 0.71	0.0706 ± 0.60

[a]IC_{50}/IC_{90} in µg/mL, after 48 h. Human cancer cell lines: THP-1 from acute monocytic leukemia, Panc-1 from pancreas carcinoma and A549 from lung adenocarcinoma. [b]Cell viability >80% at the highest concentration of 100 µg/mL for THP-1, Panc-1, A549 cells. [c]Standard anticancer drug and positive control. Data were expressed as the means of triplication. SD (±): Standard deviation.

TABLE-5
QUANTITATIVE ESTIMATES OF THE PER-RESIDUE INTERACTION ANALYSIS OF THE FLAVONOIDS FROM *A. officinarum* (**1** AND **2**) WITH DECAPRENYLPHOSPHORYL-D-RIBOSE OXIDASE (DprE1)

Compound	Docking score	Binding energy	Per-residue interaction energy analysis			
			van der Waals (kcal/mol)	Electrostatic (kcal/mol)	H-bonding (Å)	π-π stacking (Å)
1	-8.87	-40.112	Lys418 (-2.683), Cys387 (-3.390), Lys367 (-1.277), Val365 (-2.256), Leu363 (-1.247), Gln336 (-1.780), Leu317 (-2.768), Lys134 (-1.321), Gly133 (-1.210), His132 (-2.879), Gly117 (-1.538), Pro116 (-1.171), Tyr60 (-1.495)	Lys418 (-4.27), Leu363 (-2.364), Tyr60 (-2.022)	Tyr60 (2.16), Leu363 (2.38), His132 (2.60)	His132 (2.701)
2	-8.95	-41.063	Lys418 (-2.502), Cys387 (-3.258), Lys367 (-1.242), Val365 (-2.331), Leu363 (-1.223), Gln336 (-1.208), Leu317 (-2.563), Lys134 (-1.244), Gly133 (-1.106), His132 (-2.746), Gly117 (-2.430), Pro116 (-1.448), Tyr60 (-1.013)	Lys418 (-2.028), Leu363 (-3.157), Tyr60 (-1.33)	Tyr60 (2.06), Gly117 (2.41), Leu363 (2.06)	His132 (2.831)

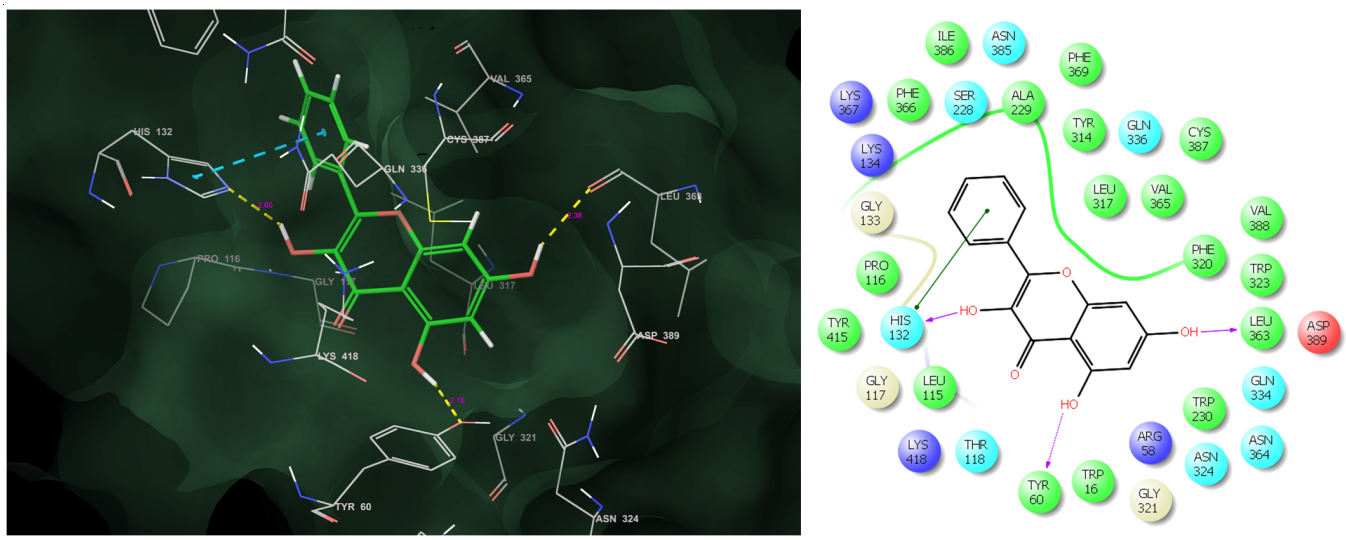


Fig. 4. Binding mode of compound **1** (galangin) into active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions)

(-41.063 kcal/mol) with the enzyme in which the contribution of the van der Waals interactions (-35.675 kcal/mol) was found to be higher than the coulombic interactions (-5.389 kcal/mol). This higher binding affinity **2** to the active site of DprE1 is attributed to the specific bonded and non-bonded per-residue interactions with the residues lining the active site. A detailed analysis of the per-residual interactions revealed that the compound is stabilized within the active site of DprE1 through an extensive network of significant van der Waals interactions with Lys418 (-2.502 kcal/mol), Cys387 (-3.258 kcal/mol), Lys367 (-1.242 kcal/mol), Val365 (-2.331 kcal/mol), Leu363 (-1.223 kcal/mol), Gln336 (-1.208 kcal/mol), Leu317 (-2.563 kcal/mol), Lys134 (-1.244 kcal/mol), Gly133 (-1.106 kcal/mol), His132 (-2.746 kcal/mol), Gly117 (-2.430 kcal/mol), Pro116 (-1.448 kcal/mol) and Tyr60 (-1.013 kcal/mol) residues through the flavone scaffold. The enhanced binding affinity of compound **2** can also be attributed to favourable electrostatic interactions observed with Lys418 (-2.028 kcal/mol), Leu363 (-3.157 kcal/mol), and Tyr60 (-1.33 kcal/mol) through the backbone. Furthermore, the compound is anchored to the active site of DprE1 through three crucial hydrogen bonding interactions *via* three-hydroxyl substitution on the flavone scaffold: 3-OH: Gly117 (2.41 Å), 5-OH: Tyr60 (2.06 Å) and 7-OH: Leu363 (2.06 Å). A very prominent π-π stacking observed *via* His132 (2.831 Å) further stabilized the ligand-enzyme complex. These types of the pi-pi stacking and H-bonding interactions serve as an "anchor", determining the 3D orientation of the molecule in the active site and also facilitate the steric and electrostatic interactions contributing to the stability of the enzyme-inhibitor complex.

This study reports the antitubercular activity of all the isolated compounds which have exhibited profound efficiency to inhibit mycobacteria in dormant and active stage. Compound **1** inhibited the growth of *M. tuberculosis* by *in vitro* and *ex vivo* with MIC/IC_{50} value of < 3 µg/mL/< 0.7 µg/mL (Table-2).

Conclusion

The bioguided extraction and isolation of marker compounds from *Alpinia officinarum* rhizomes have presented two flavonoidal antitubercular leads galangin (**1**) and kaempferide (**2**) with promising inhibition against *Mycobacterium tuberculosis*. The molecular docking of these flavones in active sites of

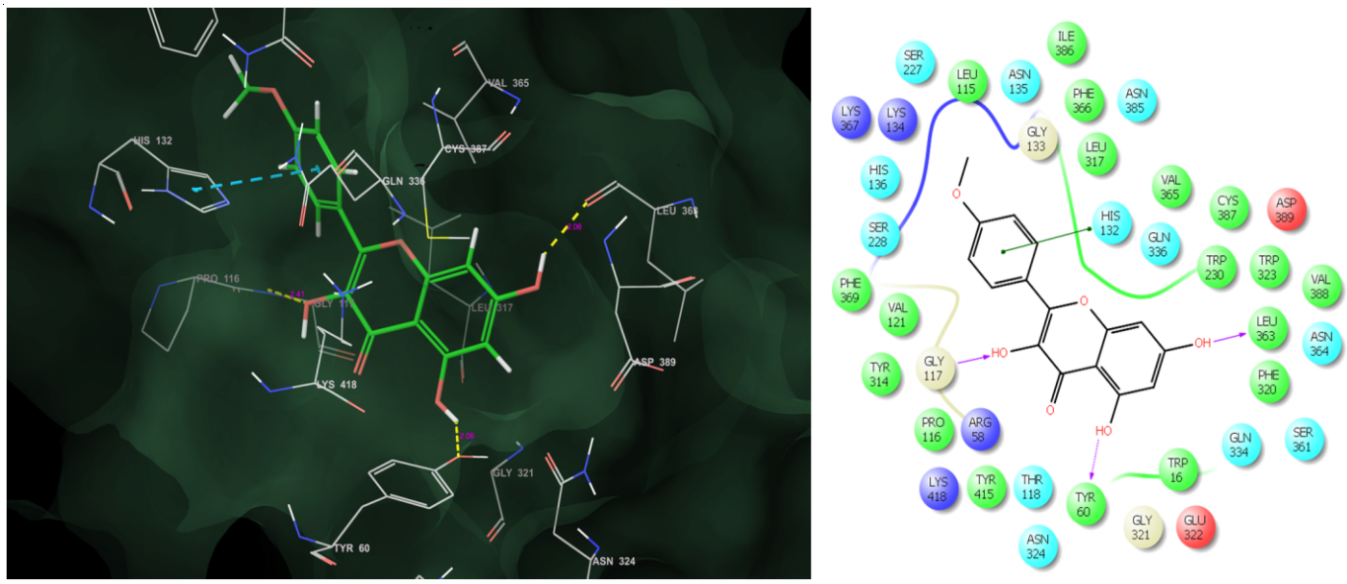


Fig. 5. Binding mode of compound **2** (kaempferide) into active site of DprE1 (on right side: green lines signify π-π stacking interactions while the pink lines represent the hydrogen bonding interactions)

DprE1 (mycobacterial target) provided critical data helped to establish the molecular basis of the interactions and explain experimental findings. Per-residue interaction analyses helped understand the thermodynamic interaction type that governed molecule binding and binding patterns in the cavity. These findings provide a platform for structure-based optimisation of the scaffolds. This study indicted satisfactory biocompatibility against human cancer cell lines and HUVECs and use of compounds and extracts acquired from *A. officinarum* which can help the future studies on novel active therapeutic compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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