Tropical Journal of Pharmaceutical Research December 2016; 15 (12): 2633-2640

ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org

http://dx.doi.org/10.4314/tjpr.v15i12.14

Original Research Article

In vivo and in silico investigation of selected herbal compounds as anti-tubercular agents

Bin Wu[1], Guang-Jun Jin[2] and Jun-Feng Chen[3]*

[1]Department of Respiratory, The Xiao Shan Hospital, [2]Department of Digestion, The First Hospital Zhejiang University School of Medicine, Hangzhou, 310000, [3]Department of Respiratory, The First People's Hospital of Wenling, Taizhou, 317500, Zhejiang, PR China

For correspondence: **Email: chenjunfeng329@hotmail.com; **Tel/Fax:** 0086-0576-86206144*

Received: 27 April 2016 *Revised accepted: 8 November 2016*

Abstract

Purpose: *To investigate whether some herbal compounds, namely, arctiin, NSC333050, cnicin, and arctigenin, can be used as anti-tubercular agents using in vivo and in silico techniques.*
Methods: *A set of structurally diverse herbal compounds were screened for anti-tubercular activity against the Mycobacterium tuberculosis (Mtb) H37v strain by determining their microbial inhibitory concentration (MIC) and cytotoxicity. The compounds were also screened using in silico techniques, such as molecular docking and absorption, distribution, metabolism, and excretion (ADME) prediction.*
Results: *The in vivo methods, such as determination of MIC and cytotoxicity assay, revealed that some of the herbal compounds showed superior anti-tubercular activity. In silico approaches involving molecular docking simulations for the mycobacterial enzymes Mtb DNA gyrase, Mtb betalactamase, Mtb diaminopelargonic acid synthase, and Mtb cytidine 5'-triphosphate synthase (CTP) confirmed that the inhibitory activities of the herbal compounds occurred at the active sites of these enzyme. In silico ADME prediction also confirmed the pharmacokinetic safety of these herbal compounds.*
Conclusion: *Arctiin, NSC333050, cnicin, and arctigenin, are suitable candidates for clinical evaluation for the treatment of respiratory infections caused by Mtb.*

Keywords: *Tuberculosis, Microbial inhibitory concentration, Arctiin, Cnicin, Arctigenin, In silico, Respiratory infection*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Tuberculosis (TB) is an infection that mainly affects the lungs, impairing respiratory function and causing other complications [1]. The symptoms include cough, fever, haemoptysiss, and weight loss [2]. TB remains prevalent in many regions [3]. *Mycobacterium tuberculosis* (Mtb) is the pathogenic bacterium that causes pulmonary tubercular infection. The number of deaths due to active TB infection has increased at an alarming rate [4,5], and it has been estimated that approximately 2 billion people are infected with latent or active Mtb worldwide [6]. In addition, new multi-drug-resistant MDR-Mtb strains and extensively drug-resistant (XDR-Mtb) strains have emerged and spread to more than 58 countries [7,8], which has complicated treatment [9]. The protocols for treating TB also take a long time and are expensive. Hence, new anti-TB drugs and better therapeutic strategies against TB are urgently needed [10,11].

In recent years, focus [12]. At present, some compounds are in clinical trial, while others are in the pre-clinical investigation stage [13]. In addition, many researchers have reported on

compounds that possess strong anti-tubercular activity, but these were not entered into clinical trials due to health-related complications [14]. In view of these concerns over health risks, this investigation focused on the *in vivo* screening of certain compounds present in herbal plants for anti-tubercular activity, which could prevent respiratory infection. It also involved *in silico* approaches such as molecular docking simulations, which revealed that the compounds inhibit certain mycobacterial enzymes. *In silico* absorption, distribution, metabolism, and excretion (ADME) prediction of compounds that dock at the active sites of the enzymes was also carried out, and it revealed that some such compounds are promising for treating TB and could potentially help in the fight against respiratory infections. These compounds have a very low risk of health-related hazards, as most of them are derived from herbal medicinal plants.

EXPERIMENTAL

Chemical compounds

A 35 herbal compounds present in herbal medicinal plants were purchased from ABI Chem (Munich, Germany) and Sigma-Aldrich (St. Louis, MI, USA). All of the commercial reagents and media were purchased from Sigma Aldrich and Hi Media (Paris, France), and the cell lines were supplied by the hospital authorizing the study. The names of the chemicals and their two-dimensional structures, PubChem IDs, and herbal plant sources are presented in Table ST1 in the supplementary material.

Determination of microbial inhibitory concentration (MIC)

The anti-mycobacterial activity was assessed by determining the MIC values against the Mtb H37Rv strain using the microbroth dilution method [15,16]. Initially, all of the 35 herbal compounds, along with isoniazid (control), were dissolved in dimethyl sulfoxide (DMSO) at 10 mg/mL and stored at 80 °C. The compound stocks were further diluted with Middlebrook 7H9 medium and adjusted to a final volume of 100 µL. The stocks were then dispensed in a 96-well round-bottomed microtitre plate using the serial dilution method. In addition, a standard log-phase bacterial suspension (MtbH37Rv strain) grown in Middlebrook 7H9 medium was prepared, to which 0.05 % Tween 80 was added at 35 °C to achieve absorbance of 0.005, corresponding to 100 colony-forming units/mL [17]. The inocula were then added to drug plates to give a total volume of 200 µL, with final drug concentrations beginning at 200 µg/mL. The plates were then sealed and incubated for 12 days at 37 °C, and the minimum concentration of the drug that inhibited bacterial growth at a rate of more than 90 % was determined. The MIC was determined as the minimum compound concentration at which the well was clear, indicating that no bacterial growth had occurred.

Determination of cytoxicity

The cytotoxicity assay was performed by culturing Vero epithelial cells (ATCC No. CCL-81) and Jurkat cells (T-lymphocyte line, ATCC No. CRL-2676) in Dulbecco's modified Eagle's medium supplemented with 5 or 10 % foetal calf serum, respectively [18]. The cell lines were grown at 37 °C in a 96-well plate in an atmosphere of 5 % CO_2. Cytotoxicity (CD_{50}), which represents the concentration that results in mortality of 50 % of the cells, was estimated by incubating the uninfected cells at concentrations in the range of 0 – 100 µg/mL for 72 h along with the herbal compounds, followed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. The MTT assay was performed as described by Mossmann (1983). First, the assay was optimised for the cell lines, incubated for 4 h with 0.8 mg/ml MTT, dissolved in serum-free medium, and washed with 1–2 ml of phosphate-buffered saline (PBS) (1 ml). This was followed by the addition of 1 ml of DMSO and shaking of the mixture for 10 min, to achieve complete dissolution. Approximately 200-µl samples of the resulting aliquots were then transferred to 96-well plates and used for measurement of absorbance at 560 nm using a microplate spectrophotometer system [19]. The compounds were then dissolved at a concentration of 40 mg/mL with DMSO as the stock solution.

Molecular docking

To check the molecular interactions of the herbal compounds with mycobacterial enzymes, molecular docking studies were carried out against proteins of the Mtb H37v strain using MVD 5.0 [20]. Specifically, the molecular docking simulations were performed against Mtbs DNA gyrase (PDB ID: 3IG0); Mtb betalactamase (MtbBlac; PDB ID: 4QHC); Mtb diaminopelargonic acid synthase, a Bio A enzyme (PDB ID: 4XEW); and Mtb cytidine 5′-triphosphate synthase synthase (CTP), a PyrG enzyme (PDB ID: 4ZDI). Initially, the potential ligand binding of these enzymes was predicted using a cavity detection program implemented in MVD 5.0. Subsequently, the protein was stabilised in accordance with the protein preparation protocol of MVD 5.0. The potential

ligand binding sites of these four enzymes were predicted using MVD 5.0. Here, the method for detecting a potential ligand binding site involves a grid-based cavity prediction algorithm, in which a discrete grid with a resolution of 0.8 Å is created and placed in a 1.4-Å-radius sphere. Whether the sphere overlaps with the protein atoms' van der Waals radii was then investigated. Then, each accessible grid point was further checked to determine whether it was part of a cavity. The final step was to determine the connected regions. The identified cavities were then ranked according to their volume [21].

The 2D structures of the 36 compounds along with isoniazid (for validation) were also retrieved from the PubChem database and converted to 3D format; then, and their geometries were optimised using HyperChem7.0 [22]. The optimised 3D structures of the herbal compounds were loaded in MVD, and their bond flexibility was set. The root-mean-square deviation threshold for multiple cluster poses was set at 2.00 Å. The docking algorithm was set at a maximum of 1500 iterations with a simplex evolution size of 50; a minimum of 50 docking simulation runs were carried out, and the best pose was chosen. Finally, ADME prediction was carried out for the compounds that docked at the active sites of the mycobacterial enzymes.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2010 to determine correlations with *in vivo* results. In addition, the energy parameters generated in the docking simulation were analysed using Molegro Data Modeller 2.0 [26]. Docking scores were ranked based on parameters such as rerank score, interaction energy, and hydrogen bond energy.

RESULTS

In vivo and *in silico* studies of 36 compounds were carried out for anti-tubercular screening against the Mtb H37v strain. These revealed that arctiin, NSC333050, cnicin, capsaicin, campesterol, sophoricoside, arctigenin, shogaol, and rhamnetin exhibited strong anti-microbial activities. In this study, the MIC was defined as the minimum concentration of the herbal compound required to induce complete inhibition of mycobacterial growth. The MIC values of these compounds were found to be lower than that of isoniazid (control), as shown in Table 1.

From screening this set of structurally diverse herbal compounds, the PubChem Bioassay database showed that most of these herbal compounds have not been subjected to anti-tubercular screening. Hence, the MIC values presented here constitute novel data. The MIC value was lowest for arctiin, at 0.64 µg/mL, while that of campesterol was 0.86 µg/ml, which is very close to the MIC value of isoniazid (0.89 µg/ml). The MIC values of gomisin A and gingerol were found to be the highest, at more than 200 µg/mL (Table 1).

Table 1: MIC values of the herbal compounds against Mtb H37Rv strain

S/N	Compound	MIC (µg/ml)	S/N	Compound	MIC (µg/ml)
1	Alliin	27	19	Eugenol	150
2	Aloe emodin	>25	20	Gingerol	<200
3	Apigenin	>25	21	Gomisin A	<200
4	Arctigenin	0.63	22	Isoniazid	0.89
5	Arctiin	0.64	23	Isorhamnetin	>50
6	Bisabolol	100	24	Kaempferol	100
7	Campesterol	0.86	25	Myricetin	>75
8	Capsaicin	0.82	26	Nordihydroguaiaretic acid	125
9	Chamazulene	100	27	NSC333050	0.71
10	Cnicin	0.76	28	Rhamnetin	7.3
11	Cycloartenol	34	29	Shogaol	10
12	Cycloastragenol	100	30	Sophoricoside	1.2
13	Daphnoretin	>25	31	Stigmasterol	>100
14	Ellagic Acid	30	32	Tetrandrine	25
15	Emodin	>25	33	Thymol	50
16	Ephedrine Hydrochloride	>25	34	Vanillin	22
17	Eugenin	50	35	Zingerone	100
18	Eugenitin	12.5			

MIC- Minimum inhibitory concentration

Table 2: Cytotoxicity of compounds with MIC lower than that of isoniazid

Compound	CD_{50} (µg/mL)	
	Vero	Jurkat
Arctiin 1	>100	100
NSC333050	>200	100
Cnicin	>100	100
Capsaicin	>400	>300
Campesterol	>200	150
Sophoricoside	>500	400
Arctigenin	>100	>200
Shogaol	>400	>100
Rhamnetin	>300	>100
Isoniazid	>400	>400

CD_{50} - represents the concentration that results in mortality of 50 % of the cells of Vero – Lineages of cells isolated from kidney epithelial cells extracted from an African green monkey; Jurkat – Immortalized cell line of human T lymphocyte cells

To understand the adverse effects of the herbal compounds, the cell cytotoxicity of those compounds with an MIC lower than that of isoniazid (0.89 µg/ml) was studied in Vero and Jurkat cell cultures. Table 2 shows the cell cytotoxicity levels of the herbal compounds, which may result in a variety of cell fates, such as necrosis and cell lysis.

Table 3 shows the molecular docking scores of the bioactive compounds present in the herbal plants against four different enzymes, namely Mtb DNA gyrase (PDB ID: 3IG0); MtbBlac (PDB ID:4QHC); Mtb diaminopelargonic acid synthase, BioA (PDB ID: 4XEW); and MtbCTP synthase, a PyrG (PDB ID: 4ZDI). The scores and results for the molecular docking simulations based on the rerank score, interaction energy, and hydrogen bonding energy are shown in Table 3. The overall docking scores revealed that arctiin, cnicin, NSC333050, and arctigenin inhibited these mycobacterial enzymes. Images depicting the molecular interactions are presented in Figure 1, Figure 2, Figure 3 and Figure 4 for the top docking hits for these enzymes. Physiochemical properties such as hydrogen bond donor, hydrogen bond acceptor, and molecular weight, which might contribute to the biological properties of these herbal compounds, are shown in Table 4. Lastly, the *in silico* ADME prediction results for the top docking hit compounds are shown in Table 5.

DISCUSSION

Screening of 34 herbal compounds revealed that arctiin, NSC333050, cnicin, capsaicin, campesterol, sophoricoside, arctigenin, shogaol, and rhamnetin possess strong anti-tubercular activities. The MIC values of these compounds were found to be better than that of isoniazid (Table 1), indicating that they possess synergistically enhanced anti-tubercular activities. Determination of the MIC against Mtb of various herbal compounds has also been reported by Lall *et al* [23]. They reported the effectiveness of herbal compounds with lower MIC values, which is consistent with the present report. Furthermore, McGaw *et al* reported the effectiveness of herbal compounds at inhibiting various mycobacterial species compared with

Table 3: Molecular docking scores for herbal compound

Ligand	Rerank Score	Interaction	HBond
PDB ID: 3IG0			
Arctiin	-89.36	-151.91	-2.99
C333050	-88.75	-145.54	-2.74
Cnicin	-86.65	-140.64	-3.54
Sophoricoside	-84.61	-140.96	-12.21
Capsaicin	-83.94	-149.81	-3.31
Gingerol	-83.64	-111.82	-5.41
Arctigenin	-80.21	-119.08	-2.40
Isoniazid	-78.10	-119.26	-2.12
Cycloartenol	-75.80	-122.25	-0.73
PDB ID: 4XEW			
Arctiin	-99.45	-156.04	-8.92
NSC333050	-95.57	-110.39	-4.98
Stigmasterol	-92.44	-122.15	-2.50
Cnicin	-90.52	-114.19	-10.46
Arctigenin	-92.04	-127.15	-9.75
Campesterol	-91.40	-129.44	-0.82
Isoniazid	-91.24	-143.25	-2.67
Rhamnetin	-83.75	-123.06	-10.91
Shogaol	-79.21	-104.11	-5.09
PDB ID: 4XEW			
Arctiin	-107.85	-163.23	-13.58
Cnicin	-103.61	-118.98	-13.42
Arctigenin	-98.98	-130.43	-4.11
NSC333050	-95.00	-108.88	-1.85
Capsaicin	-92.41	-133.23	-0.77
Sophoricoside	-90.82	-128.54	-17.26
Cycloastragenol	-90.05	-118.87	-9.19
Campesterol	-89.77	-127.50	-4.46
Stigmasterol	-85.69	-123.85	0.00
PDB ID: 4ZDI			
Arctiin	-107.39	-156.90	-5.40
Arctigenin	-103.41	-141.52	-11.21
NSC333050	-103.13	-130.31	-9.27
Sophoricoside	-92.73	-151.46	-9.33
Stigmasterol	-90.93	-121.72	0.00
Rhamnetin	-89.88	-138.08	-8.93
Cnicin	-88.12	-116.53	-6.05
Isoniazid	-87.38	-119.88	-2.00
Cycloartenol	-87.06	-129.78	0.00

Rerank score - The rerank score is a linear combination of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between the ligand and the protein, and E-intra; **Interaction** - The total interaction energy between the pose and the protein (kJ mol^{-1}); **Hbond** - Hydrogen bonding energy (kJ mol^{-1})

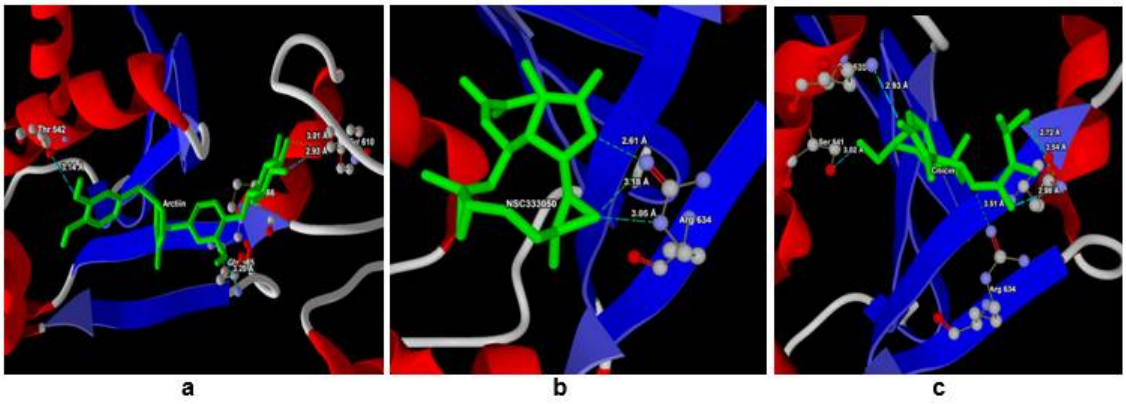

Figure 1: Molecular interaction of (a) Arcitin with Thr542, Gln565, Tyr610 residues (b) NSC333050 with Arg634 and (c) Cnicin with Gln538, Ser541, Gln565, Arg634 residues of 3IGO

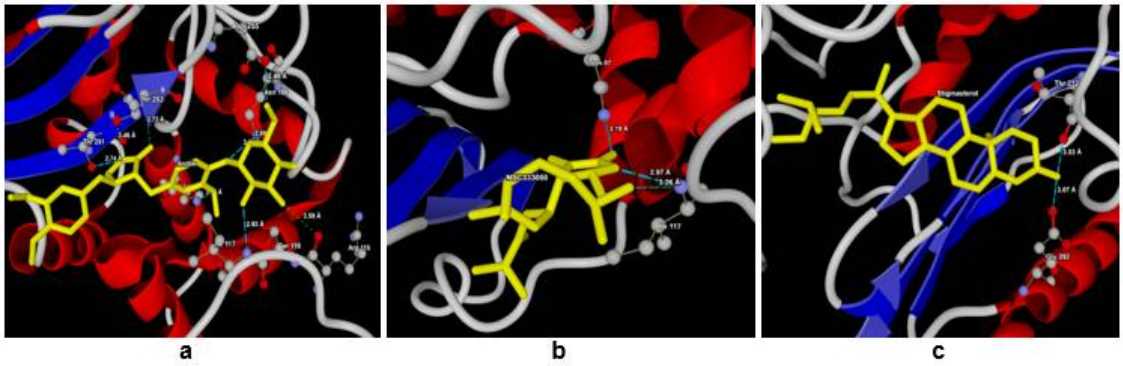

Figure 2: Molecular interaction of (a) Arctiin with Arg115, Ile117, Asp255, Asn186, Thr253, Thr251, Lys87 (b) NSC333050: Ile117, Lys87 (c) Stigmasterol with Glu292, Thr232 residues of 4QHC

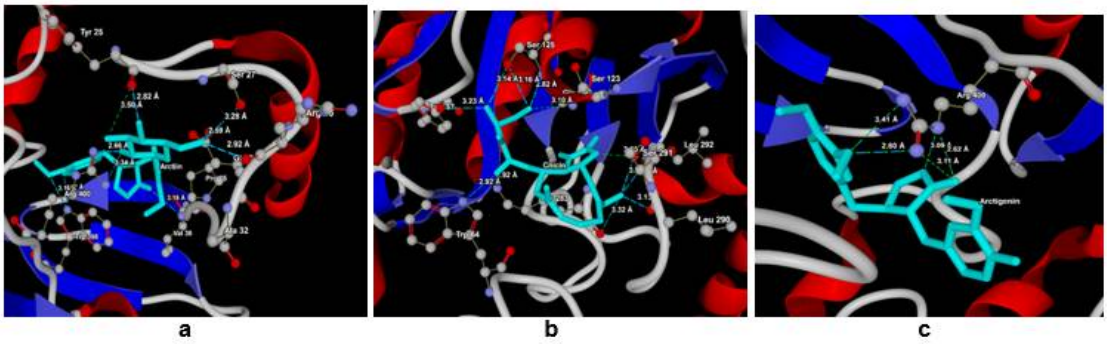

Figure 3: Molecular interaction of (a) Arctiin with Tyr25, Ser27, Arg30, Glu31, Val36, Trp398, Arg400 (b) Cnicin with Trp64, Gly124, Ser125, Tyr157, Lys283, Leu290, Leu292 (c) Arctigenin with Arg400 residues of 4XEW

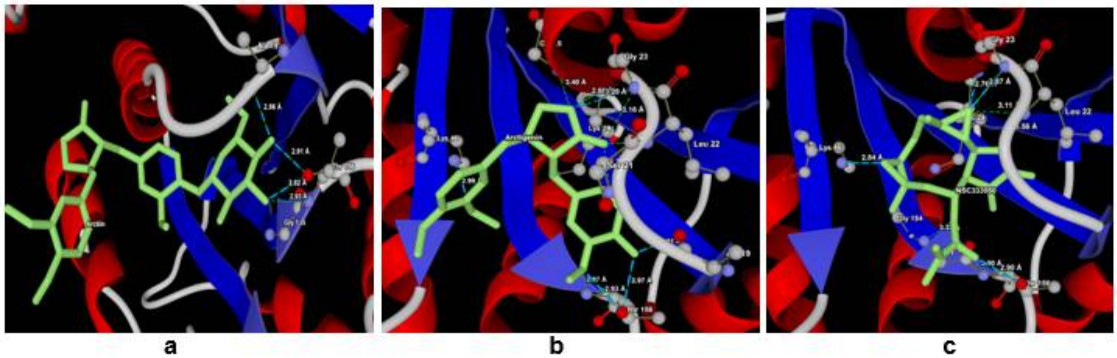

Figure 4: Molecular interaction of (a) Arctiin with Ala19, Thr156 (b) Arctigenin with Ala19, Ser21, Leu22, Gly23, Lys24, Gly25, Lys46, Thr156 (c) NSC333050 with Leu22, Gly23, Lys24, Lys46, Gly155, Thr156 residues of 4ZDI

Table 4: Physiochemical properties of arctiin, NSC333050, cnicin, arctigenin and isoniazid

Compound	MW	HBA	HBD	XLog P3	Rot B	TPSA (Å^2)
Arctiin	534.55	11	4	1.8	10	153
NSC333050	348.39	6	0	2.3	3	77.7
Cnicin	378.41	3	7	0.2	6	113
Arctigenin	372.41	6	1	3.6	7	74.2
Isoniazid	137.13	3	3	-0.7	1	68.0

MW – Molecular weight; **HBA** – Hydrogen bond acceptor; **HBD** – Hydrogen bond donor; **XLog P3 –** Computed octanol-water partition coefficient; **Rot B** – Number of rotatable bonds; **TPSA** - Topological polar surface area

Table 5: *In silico* ADME prediction for arctiin, NSC333050, cnicin, arctigenin and isoniazid

Compound	Absorption (%)	Absorption rate (min^{-1})	Volume of distribution (L/kg)	Oral bioavailability (%)
Arctiin	**97**	0.009	**1.02**	**30**
NSC333050	100	0.053	**1.46**	**30**
Cnicin	**100**	0.034	**1.11**	30-70
Arctigenin	100	0.051	**1.52**	**30**
Isoniazid	**90**	0.005	**0.9**	**<70**

synthetic drugs available on the market [24]. Among the nine most active herbal compounds, none of the compounds was properly investigated for its anti-tubercular activity in previous studies. Hence, the MIC values presented here constitute novel data on these compounds.

The cell cytotoxicity tests of these compounds in this study showed that arctiin, cnicin, and arctigenin have lower cell toxicity levels than the rest of the tested compound, with CD_{50} values of less than 100 µg/mL (Table 2) in both Vero and Jurkat cell cultures. Cytotoxicity assays were performed for the compounds with MIC values lower than that of isoniazid. The CD_{50} values of the tested compounds in Vero cells were lower than the toxicity level of the drug isoniazid, which has been approved for public use (Table 2). The CD_{50} values of the tested herbal compounds demonstrate that they have a good safety profile compared with isoniazid.

The scoring function for molecular docking studies carried out using MVD 5.0 (Table 3) is based on the modified piecewise linear potential with new hydrogen bonding and electrostatic terms [27]. In these studies, only the top three docking hits were considered based on the rerank scores, which is a linear combination of E-inter (including van der Waals, steric, hydrogen bonding, and electrostatic forces) between the ligand and the protein, and E-intra (including van der Waals, hydrogen bonding, torsion, sp2-sp2, and electrostatic forces of the ligand) weighted by pre-defined coefficients [27].

In the first simulation carried out against Mtb DNA gyrase (PDB ID: 3IG0), arctiin, NSC333050, and cnicin inhibited the active site of the enzyme, with rerank scores of −89.36, −88.75, and −86.65 kJ mol^{-1}, respectively (Table 3: 3IG0). In the second simulation performed against MtbBlac (PDB ID: 4QHC), arctiin, NSC333050, and stigmasterol inhibited this enzyme with rerank scores of −99.45, −95.57, and −92.44 kJ mol^{-1}, respectively (Table 3: 4QHC). In the third simulation, arctiin, cnicin, and arctigenin inhibited diaminopelargonic acid synthase, a BioA enzyme (PDB ID: 4XEW), with rerank scores of −107.85, −103.61, and −98.98 kJ mol^{-1}, respectively (Table 3: 4XEW). Lastly, arctiin, arctigenin, and NSC333050 inhibited CTP synthase, a PyrG enzyme (PDB ID: 4ZDI), with rerank scores of −107.39, −103.41, and −103.13 kJ mol^{-1}, respectively (Table 3: 4ZDI). Images of the molecular interactions are shown in Figures 1–4, where the interacting amino acids are highlighted. In the present investigation, the most promising herbal compounds with regard to anti-tubercular activity based on MIC values, cell cytotoxicity assay, and molecular docking score are arctiin, NSC333050, cnicin, and arctigenin. These inhibitors displayed considerable *in vitro* and *in silico* anti-tubercular activities.

Among the investigated Mtb enzymes, arcitiin interacts with the Thr542, Gln565, and Tyr610 residues of Mtb DNA gyrase; the Arg115, Ile117, Asp255, Asn186, Thr253, Thr251, and Lys87 residues of MtbBlac; the Tyr25, Ser27, Arg30, Glu31, Val36, Trp398, and Arg400 residues of Mtb diaminopelargonic acid synthase; and the Ala19 and Thr156 residues of Mtb CTP. This reflects the fact that arcitiin has the most potent anti-tubercular activity. The increased anti-tubercular activity of these compounds compared with that of isoniazid may be due to the increased numbers of hydrogen bond donors and acceptors of these compounds (Table 4). Statistical analysis using Pearson's correlation revealed a weak correlation between the MIC

values and the CD_{50} for Vero cells (r = 0.345), whereas no significant correlation was established for Jurkat cells (r = −0.358). However, an intermediate correlation was established between the CD_{50} values of Vero and Jurkat values (r = 0.671).

In addition, *in silico* ADME absorption, distribution, metabolism, and excretion predictions revealed the favourable pharmacokinetic behaviour of these herbal compounds regarding their disposition in the human body (Table 5). The results show that these herbal compounds may be promising for the development of drugs for the treatment of infections caused by Mtb.

CONCLUSION

Arctiin, NSC333050, cnicin, and arctigenin are promising anti-tubercular agents, based on the results of *in vivo* and *in silico* investigations. As these compounds are constituents of herbal medicinal plants, they have a low risk of side effects. Thus, it seems that these compounds are suitable candidates for clinical studies.

DECLARATIONS

Acknowledgement

The authors would like to acknowledge the authority of Xiao Shan Hospital, Hangzhou, The First Hospital Zhejiang University School of Medicine, Hangzhou, Zhejiang China and The First People's Hospital of Wenling, Taizhou, Zhejiang, China for providing the facility to carry out this investigation.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

REFERENCES

1. *Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med 2007; 13(5): 175-182.*
2. *Ouellet H, Johnston JB, de Montellano PR. Cholesterol catabolism as a therapeutic target in Mycobacterium tuberculosis. Trends Microbiol 2011; 19(11): 530-539.*
3. *Pearson A, Budin M, Brocks JJ. Phylogenetic and biochemical evidence for sterol synthesis in the bacterium Gemmataobscuriglobus. Proc Natl Acad Sci USA. 2003; 100(26): 15352-15357.*
4. *Flynn JL, Chan J. Immune evasion by Mycobacterium tuberculosis: living with the enemy. Curr Opin Immunol 2003; A15(4): 450-455.*
5. *Lawn SD. Tuberculosis and HIV co-infection. Medicine. 2009; 37(12): 654-656.*
6. *Check E. After decades of drought, new drug possibilities flood TB pipeline. Nat Med 2007; 13 (3):266.*
7. *Raviglione MC, Smith IM. XDR tuberculosis-implications for global public health. N Engl J Med 2007; 356(7): 656-659.*
8. *Mowa MB, Warner DF, Kaplan G, Kana BD, Mizrahi V. Function and regulation of class I ribonucleotide reductase-encoding genes in mycobacteria. J Bacteriol 2009; 191(3): 985-995.*
9. *Lienhardt C, Glaziou P, Uplekar M, Lönnroth K, Getahun H, Raviglione M. Global tuberculosis control: lessons learnt and future prospects. Nat Rev Microbiol 2012; 10(6): 407-416.*
10. *Morisseau C, Hammock BD. Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. Annu Rev Pharmacol Toxicol 2005; 45: 311-333.*
11. *Bini EI, Hernandez-Pando R. New Chemotherapy and Immunotherapy for Tuberculosis. Curr Resp Med Rev 2014; 10(2): 74–87.*
12. *Swindell S: New drugs to treat tuberculosis. F1000 Med Rep 2012; 4: 12.*
13. *Kamal A, Azeeza S, Malik MS, Shaik AA, Rao MV. Efforts towards the development of new antitubercular agents: potential for thiolactomycin based compounds. J Pharm Pharm Sci 2008; 11(2): 56s-80s.*
14. *North EJ, Jackson M, Lee RE. New approaches to target the mycolic acid biosynthesis pathway for the development of tuberculosis therapeutics. Curr Pharm Des 2014; 20(27): 4357–4378.*
15. *Kobayashi I, Muraoka H, Saika T, Nishida M, Fujioka T, Nasu M. Micro-broth dilution method with air-dried microplate for determining MICs of clarithromycin and amoxycillin for Helicobacter pylori isolates. J Med Microbiol 2004; 53(5): 403-406.*
16. *Halbert LW, Kaneene JB, Mansfield LS, Ruegg PL, Warnick LD, Wells SJ, Fossler CP, Campbell AM,*

Geiger-Zwald AM. Comparison of automated microbroth dilution and agar dilution for antimicrobial susceptibility of Campylobacter jejuni isolated from dairy sources. J Antimicrob Chemother 2005; 56(4): 686-691.

17. *Ronald MA, Snyder JW. Handbook of media for clinical microbiology. CRC Press, 2006.*
18. *Decker T, Lohmann-Matthes ML.A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. Immunol Methods 1988; 115(1): 61-69.*
19. *Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 16: 55-63.*
20. *MVD 5.0 – Molegro Virtual Docker, Denmark.*
21. *Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. J Med Chem 2006; 49: 3315-3321.*
22. *Hyperchem 7.0, Hypercube Inc, FL, USA.*
23. *Lall N, Meyer JJ, Wang Y, Bapela NB, van Rensburg CEJ, Fourie B, Franzblau SG. Characterization of intracellular activity of antitubercular constituents from the roots of Euclea natalensis. Phar Biol 2005; 43: 353-357.*
24. *McGaw LJ, Lall N, Hlokwe TM, Michel AL, Meyer JJ, Eloff JN. Purified compounds and extracts from Euclea species with antimycobacterial activity against Mycobacterium bovis and fast-growing mycobacteria. Biol Pharm Bull. 2008; 31(7): 1429-1433.*