Pharmaceutical Sciences, 2020, 26(4), 441-447
doi:10.34172/PS.2020.38
https://ps.tbzmed.ac.ir/

Research Article

Antibacterial Components of *Levisticum officinale* Koch against Multidrug-resistant *Mycobacterium tuberculosis*

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Article Info

Article History:
Received: 26 January 2020
Accepted: 18 May 2020
ePublished: 25 December 2020

Keywords:
-Antibacterial activity
-Bioassay-guided fractionation
-*Levisticum officinale*
-Molecular docking
-Multidrug resistance
-*Mycobacterium tuberculosis*

Abstract

Background: A bioassay-guided fractionation technique was used to evaluate the active constituents of the perennial plant *L. officinale* W.D.J. Koch (Apiaceae) against multidrug resistant (MDR) *Mycobacterium tuberculosis*.

Methods: Column chromatography was used to isolation of compounds from *L. officinale* and spectroscopic methods including 1D and 2D NMR (Nuclear magnetic resonance) and HRMS (high resolution mass spectrometry) were used to identification of the isolated compounds. Also, to evaluate antibacterial activity, minimum inhibitory concentration (MIC) was carried out by broth micro-dilution method. Finally, molecular docking (MD) was performed using the Schrödinger package to evaluate interactions between the active compounds and InhA protein.

Results: Phytochemical analysis of the ethyl acetate extract of the plant roots led to isolation of bergapten (**1**), isogosferol (**2**), oxypeucedanin (**3**), oxypeucedanin hydrate (**4**), imperatorin (**5**), ferulic acid (**6**) and falcarindiol (**7**). Falcarindiol and oxypeucedanin indicated a moderate activity on MDR *M. tuberculosis* with MIC values of = 32 and 64 µg/mL, respectively. Antibacterial activity of falcarindiol was also observed against *S. aureus* and methicillin-resistant *S. aureus* strains with the MIC values of 7.8 and 15.6 µg/mL, respectively. The results of docking analysis showed a good affinity of oxypeucedanin (**3**) and falcarindiol (**7**) to InhA enzyme with docking score values of -7.764 and -7.703 kcal/mol, respectively.

Conclusion: Finally, 7 compounds were isolated from *L. officinale* that compounds 2-6 report for the first time from this plant. On the basis of the molecular docking (MD) study, oxypeucedanin (**3**) and falcarindiol (**7**) as active compounds against *M. tuberculosis* may be proposed as potential inhibitors of 2-trans-enoyl-ACP reductase (InhA), a key enzyme involved in the biosynthesis of the mycobacterial cell wall. Moreover, antibacterial activity of falcarindiol against methicillin-resistant *S. aureus* (MRSA) was remarkable.

Introduction

Tuberculosis (TB) kills about two million people every year. This dangerous infectious disease is caused by several *Mycobacterium* strains, in particular *M. tuberculosis*.[1] As a long period of time is often required for treatment, the hepatotoxicity of drugs and development of multidrug-resistance (MDR) and extreme drug resistance (XDR) are considerable complications. Tuberculosis is currently a major public health problem.[2,3] The currently available drugs for the treatment of TB are limited and in some case outdated, so discovery of new anti-TB drugs, especially against resistant strains is vital and necessary.[4] Natural products are important sources for antibacterial compounds, with around 70% of antibiotics used in the clinic derived from natural origins.[5] Among the many plants studied as a source of drug leads, *L. officinale* is an important medicinal plant due to its broad range bioactive metabolites.

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L. officinale belongs to the Apiaceae family, growing wild in most parts of the world including Asia and Europe. *L. officinale* is referred to in English as lovage and in the Persian language as Anjedan e roomi, and was collected for this study from the Hezar mountain of the Kerman province in Iran.[6,7] *L. officinale* shows various biological effects including diuretic, spasmolytic, and carminative effects, and is also used for the treatment of urinary tract infections.[8,9] Coumarins, polyacetylenes, and phthalides are some of the important compound classes found in *L. officinale*.[6,8] One of the most important biological activities reported to date for *L. officinale* is the antibacterial activity of polyacetylenes such as falcarindiol and falcarinol.[10]

Moreover, *L. officinale* is also known to be effective on several *Mycobacterium* strains such as *M. fortitum*, *M. aurum* and *M. bovis*.[8,11]

Computational strategies have been widely used in pharmacology field, to rapidly identify the new lead compounds and inhibitors of enzymes.[12] The molecular docking analysis, which is able to biologically distinguish the active components from a set of different ligands through the evaluation of protein ligand interactions, have been considered by researchers in drug design methodologies in recent years.[13] A large set of docking softwares and programs such as Autodock vina, Glide, Gold and Surflex is carried out for prediction of the behavior of various ligands with target enzymes.[14] In this research, the Glide module of Schrödinger package was used for docking analysis.

Herein, we have investigated different polarity extracts of both the aerial parts and roots of *L. officinale* and isolated compounds such as the coumarin oxypeucedanin and polyacetylene falcarindiol, which were found to be active against MDR *M. tuberculosis,* as well as *Staphylococcus aureus* and MRSA.

Materials and Methods

Plant materials

The roots of *L. officinale* were collected in 2015 from the Hezar Mountain of Kerman province, Iran. The plant material was identified by Prof. Farideh Attar. A voucher specimen (46553-TUH) has been deposited in the herbarium of the Science Faculty of Tehran University, Iran.

Extraction and isolation

The roots of *L. officinale* (3 kg) were powdered and extracted with *n*-hexane (3 x 9 L) and then ethyl acetate (3 x 9 L) by the maceration at room temperature. The ethyl acetate extract was concentrated under reduced pressure, to afford 100 g of dried extract. This extract was fractionated by silica gel-column chromatography (CC) (230- 400 mesh, 1kg), eluting as a gradient from 100% *n*-hexane to 100% ethyl acetate, followed by increasing concentration of methanol (up to 20%) in ethyl acetate. The eluents were combined to give 16 fractions based on TLC (Thin-layer chromatography) patterns. Fraction 7 (2 g) was subjected to a silica gel column chromatography (230-400 mesh), eluted with isocratic $CHCl_3$-hexane-acetone (40-50-10), to give seven subfractions F7a-F7g. From these, falcaridiol **7** (400 mg, as a light brown oil), bergapten **1** (3mg) and ferulic acid **6** (4 mg) were obtained from F7a. Subfraction F7b afforded oxypeucedanin **3** (4.4 mg) as a colorless solid after recrystallization from $CHCl_3$. Fraction 9 (4 g) was separated on silica gel CC (230-400 mesh) with a gradient of *n*-hexane-ethyl acetate to afford five subfractions F9a-F9d. Subfraction F9a (44mg) was applied to reverse phase silica gel column (6 g) and eluted with methanol-water as gradient, to afford oxypeucedanin hydrate **4** (6 mg). Subfraction F9b (85 mg) was also subjected on reversed phase silica gel (13 g) and washed with a gradient of methanol-water, which led to the isolation of isogosferol **2** (3 mg). Subfraction F9c (80 mg) was purified on reverse phase silica to give 8 subfractions F9c1-F9c8. Subfraction F9c1 was recrystallized in $CHCl_3$ to afford imperatorin **5** (4.5 mg).

Mycobacterium activity

Bacterial strains

A MDR *M. tuberculosis* strain was obtained from the microbial collection of Department of Medical Microbiology, Tehran University of Medical Sciences, Tehran, Iran. The antimicrobial susceptibility testing was done according to the Centre for Disease Control and Prevention (CDC) standard method for MDR confirmation of *M. tuberculosis*.[15,16]

Determination of MIC

Minimum inhibitory concentration (MIC) values were determined using a broth microdilution method in 96-well microtitre plates according to standard methods.[17] All wells were filled with 100 µL of Middlebrook 7H9 medium (Difco™; Becton Dickinson & Co., Sparks, MD) supplemented with glycerol and oleic acid, albumin, dextrose, and catalase (OADC, Difco™; Becton Dickinson & Co.). The first column on the plate also received 80 µL of supplemented Middlebrook 7H9 medium and 100 µL of each compound at 10 mg/mL concentration. The suspensions were diluted in seven serial dilution. Using a multichannel pipette, 100 µL was transferred from column 1 to the next column, and identical serial 1:2 dilutions were continued through to column 7.[18] The final concentration of essential oils in each row was 200-0.015 µg/mL. Each well was inoculated with 5 µl of 0.5 McFarland standard turbidity of bacterial suspensions.[17] A column without compound was inoculated as the growth control. A well with 80 µl of supplemented Middlebrook 7H9 medium and 20 µL of Dimethyl sulfoxide (DMSO) alone was also inoculated in each row to assess any for refuse anti-mycobacterial effect of DMSO. The sealed plate was incubated at 37°C for 4 weeks. The wells were evaluated after 7, 14, 21, and 28 days and they were compared with the control wells. MIC was defined as the lowest compound concentration that exhibited no growth by

visual inspection.[17] Each experiment was done in duplicate at least twice.

Antibacterial activity

Bacterial strains

In vitro antibacterial activity of extracts and compounds were assessed against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* (Vancomycin-resistant clinical strain) as Gram-positive bacteria and *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* PTCC1430 as Gram-negative bacteria. Strain bacteria were kindly provided by Professor M.M. Feizabadi, Tehran University of Medical sciences. All of the strains were confirmed by standard tests.

Determination of MIC

Minimum inhibitory concentration (MIC) values of bacterial strains were determined by broth micro dilution according to CLSI guideline using sterile 96 wells plates.[19] Each sample was serially diluted in from 256-0.015 µg/mL concentrations. Freshly bacterial suspensions were adjusted to 0.5 McFarland standards and were further diluted (1:100) using MHB medium. The microplates incubated for 24 h at 37°C. Then, lowest concentrations which could inhibit visible growth of bacterial strains was considered as MIC. Each test was done as triplicate. The Cefixime and chloramphenicol were used as the standard antibacterial agent. Each experiment was done in duplicate at least twice.

Molecular docking study

Molecular docking (MD) analysis was performed on the Schrödinger package 2016-2 (Schrödinger, LLC)[20] to evaluate the binding modes of active ligands to InhA protein using the Glide application.[18] The structure of InhA protein (PDB ID: 1BVR) was obtained from the RCSB Protein Data Bank (PDB). The 3D structure of enzyme was edited on the protein preparation wizard where, the removed H atoms were added to the structure and all water molecule and co-crystalized ligands were removed. The active site of InhA was constituted at special residues of enzyme by following coordinates: X=12.832, Y=16.388, Z= 6.306. Ligand structures were generated on ChemDraw and structurally optimized on LigPrep module to prepare the low energy 3D conformers for docking analysis. The structures were also energetically minimized in a cut off RMSD of 0.3 Å. Finally, all ligands were docked into the InhA on the Glide docking module of Schrodinger package.

Results and Discussion

In our previous studies, the antibacterial activity of the essential oils of *L. officinale* has been investigated[22,23]. Here, we report the anti-TB activity of different extracts of *L. officinale*.

In order to isolate anti-TB agents from *L. officinale*, the hexane, ethyl acetate and methanol extracts from the aerial parts and roots of *L. officinale* were investigated against MDR *M. tuberculosis*. The ethyl acetate extract of the plant roots was found to be the most active extract (Table1), from which seven known compounds including five coumarins, an acidic compound and a polyacetylene compound were isolated.

Table 1. Results of activity of *L. officinale* extracts and isolated compounds against MDR *M. tuberculosis*.

Extract and compounds	MIC (µg/mL)
Hexane (aerial parts)	512
Hexane (root)	512
Ethyl acetate (aerial parts)	256
Ethyl acetate (root)	128
Methanol (aerial parts)	>512
Methanol (root)	>512
Bergapten **(1)**	>128
Isogosferol **(2)**	>128
Oxypeucedanin **(3)**	64
Oxypeucedanin hydrate **(4)**	>128
Imperatorin **(5)**	>128
Ferulic acid **(6)**	>128
Falcarindiol **(7)**	32
Isoniazid	4

Coumarins and polyacetelynes are two important classes in Apiaceae family that we report in this study. The structures of compounds were identified as bergapten (**1**), isogosferol (**2**), oxypeucedanin (**3**), oxypeucedanin hydrate (**4**), imperatorin (**5**), ferulic acid (**6**) and falcarindiol (**7**) by extensive 1D and 2D NMR spectral optical, and HRMS data as well as by comparison with literature data[24-29] (Figure 1).

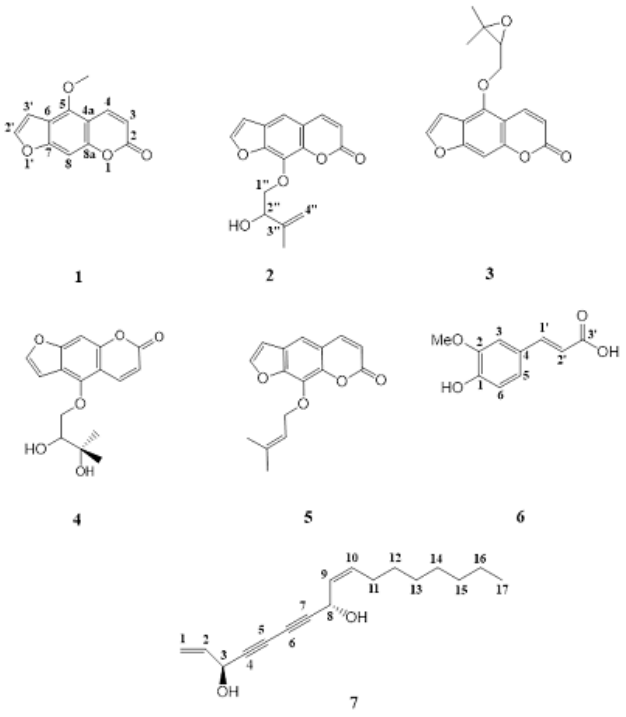

Figure 1. The structures of isolated compounds from the roots of L. officinale

Compounds **2-6** are reported for the first time from *L. officinale*. Although coumarin compounds such as umbelliferone, psoralen, apterin and bergapten were previously reported from *L. officinale*,[6,8] there is no previous report about the presence of acidic compounds and therefore the presence of ferulic acid in *L. officinale* is notable.

The activity of the isolated compounds was studied against MDR *M. tuberculosis*. Compounds **3** and **7** were relatively active with the MIC values of 64 and 32 µg/mL, respectively (Table 1). Oxypeucedanin showed a higher activity than other coumarins that have been reported here. In order to investigate the mechanism of action of compounds **3** and **7**, a molecular docking study was performed against an important enzyme involved in *M. tuberculosis* cell wall biogenesis, namely 2-trans-enoyl-ACP reductase (InhA). Isoniazid as an anti-Tuberculosis (TB) drug which inhibits InhA, was used herein as a standard ligand. The docking score values of -7.764 and -7.703 kcal/mol were recorded for compounds **3** and **7,** respectively. Molecular docking analysis showed a high affinity of the above-mentioned compounds to MDR *M. tuberculosis* in comparison with isoniazid with a docking score value of -6.013 kcal/mol (Table 2). Figure 2 shows interactions of compounds 3 and 7 with the InhA receptor, whereupon compound 3 is linked to the active site of enzyme through a hydrogen bond interaction with ILE 194. Also, Figure 2 shows two hydrogen bond interactions between compound **7** and residues ILE 21 and ILE 194 of the receptor.

The seven isolated compounds were also evaluated for antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli,* and *Pseudomonas aeruginosa* strains. Falcarindiol was found to be the most active compound against *S. aureus*, MRSA and *E. coli* with MIC values of 7.28, 15.6 and 128 µg/mL, respectively. The next most active compound was imperatorin (**5**), which showed moderate activity against *S. aureus*, and *E. coli* with a MIC values of 64 and 128 µg/mL, respectively (Table 3).

Coumarins are an important natural product class, which exhibit a wide range of pharmacological activities including antibacterial, anticancer, anticoagulant, antioxidant, antifungal, anti-inflammatory activities.[30]

Table 2. Predicted binding free energies and details of interactions of compounds 3, 7 and isoniazid with Mycobacterium tuberculosis enoyl-ACP reductase (InhA).

Compounds	Protein name	Binding energy (kcal/mol)	Interactions with amino acid residues
3	InhA	-7.764	MET147, ALA191, ILE194, PRO193, MET199,
7	InhA	-7.703	ILE21, ILE215, TYR158, ILE215, ALA157,
Isoniazid	InhA	-6.013	ARG 225, ASP150, ARG153

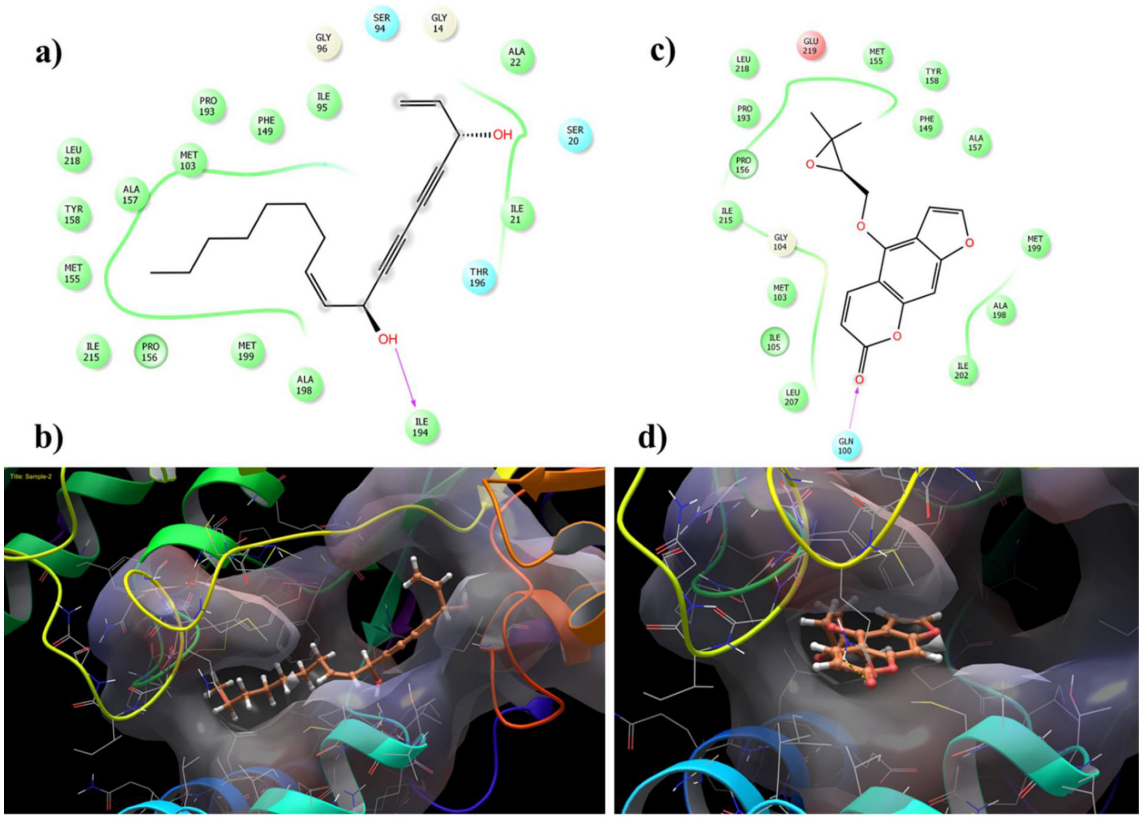

Figure 2. The predicated interaction in 2D and 3D representation for falcarindiol 7 (a and b) and oxypeucedanin 3 (c-d) with Mycobacterium tuberculosis enoyl-ACP reductase (InhA)

Table 3. Antibacterial activity of *L. officinale* metabolites against four bacterial strains.

Compounds	S. aureus	E. coli	E. faecium	P. aeruginosa	MRSA
	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC(µg/mL)
1	256	256	256	>256	>256
2	256	256	256	>256	>256
3	256	256	256	>256	>256
4	128	256	256	>256	>256
5	64	128	256	>256	>256
6	>256	>256	>256	>256	>256
7	7.8	128	256	>256	15.6
Cefixime	2	4	16	64	-
Chloramphenicol	8	32	64	>256	-

These compounds are also interesting due to their anti-TB effects. For example, the coumarin scopoletin isolated from the plant *Fatoua pilosa* showed anti-TB activity with a MIC value of 42.1 µg/mL.[31] Umbelliferone, which exist in many plants of the Apiaceae family showed moderate activity against *M. tuberculosis* with a MIC value of 58.3 µg/mL.[6,27] The coumarins xanthyletin, phellodenol A and (+)-(*S*)-marmesin isolated from the leaves of *Phellodendron amurense* var. *wilsonii* have anti-TB activity with a MIC value of 60 µg/mL.[27] Previous phytochemical investigations of *L. officinale* have also identified falcarindiol, falcarinol, and dehydrofalcarindiol as metabolites, with falcarindiol active against *M. fortuitum* and *M. aurum* with MIC values of 30.7 µM and 61.4 µM, respectively.[32] Likewise, plant-derived polyacetylene compounds were also found to be active against *M. tuberculosis* with (9*Z*,17)-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate having a MIC value of 1.4 µg/mL.[33]

Other reported, coumarin compounds have shown only low antibacterial activity against bacterial strains, for example 6-methylcoumarin, 6-methoxycoumarin, 6-aminocoumarin, 7-methoxycoumarin, 7-methylcoumarin, and 7-*O*-acetylcoumarin were tested against *B. cereus*, *E. coli*, *P. aeruginosa* and *S. aureus* giving MIC values ranging between 500-2000 or even more than 2000 µg/ml.[34] Some coumarins have shown moderate antibacterial activity such as osthenol showed antibacterial activity with a MIC value of 62.5 µg/mL against both *S. aureus* and *B. cereus*.[34] These results suggest that the presence of a non-polar short chain at position C-7 of coumarin compounds derivatives may increase the antibacterial activity, compared to a polar chain in this position decrease the antibacterial activity. Conversely, substitution at the C-5 position on coumarins may decrease antibacterial activity.

Previous reports on the antibacterial activity of plant-derived polyacetylene compounds support our results herein. Falcarinol is known to exhibit antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subitilis* and *C. albicans* with MIC values ranging between 3.1–6.3 µg/mL, while the structurally related oplopandiol was active against *E. coli*, *S. aureus*, *B. subitilis*, and *C. albicans* with MIC values between 6.3-12.5 µg/mL.[35]

Bergapten (**1**) - white amorphous powder (3 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.30 (3H, s, O-CH_3), 6.37 (1H, d, H-3), 6.82 (1H, d, H-3'), 7.35 (1H, s, H-8), 7.69 (1H, d, H-2'), 7.76 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 62.6 (O-CH_3), 106.7 (C-3'), 112.9 (C-8), 114.9 (C-3), 116.4 (C-4a), 126.2 (C-6), 142.6 (C-8a), 144.3 (C-4), 146.6 (C-2'), 147.7 (C-7), 161.4 (C-2).

Isogosferol (**2**) - white crystals (3mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.83 (3H, s, 3″-Me), 4.32 (1H, t, H-1″a), 4.55 (1H, bd, H-2″), 4.59 (1H, dd, H-1″b), 5.00 (1H, s, H-4″a), 5.17 (1H, s, H-4″b), 6.39 (1H, d, H-3), 6.84 (1H, d, H-3'), 7.41 (1H, s, H-5), 7.70 (1H, d, H-2'), 7.79 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 19.0 (3″-Me), 73.8 (C-2″), 77.1 (C-1″), 106.7 (C-3'), 113.0 (C-4″), 113.7 (C-5), 114.7 (C-3), 116.5 (C-4a), 126.1 (C-6), 131.6 (C-8), 142.7 (C-3″), 143.4 (C-8a), 144.5 (C-4), 146.9 (C-2'), 148.1 (C-7), 160.5 (C-2).

Oxypeucedanin (**3**) - colorless amorphous powder (4.4mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.34 (3H, s, Me-3″β), 1.41(3H, s, Me-3″α), 3.22 (1H, m, H-2″), 4.44 (1H, dd, H-1″a), 4.60 (1H, dd, H-1″b), 6.32 (1H, d, H-3), 6.96 (1H, d, H-3'), 7.20 (1H, s, H-8), 7.62 (1H, d, H-2'), 8.21 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 19.0 (3″β-Me), 24.7 (3″α-Me), 58.2 (C-3″), 61.2 (C-2″), 72.1 (C-1″), 94.8 (C-8), 104.5 (C-3'), 110.7 (C-4a), 113.2 (C-3), 114.2 (C-6), 138.9 (C-4), 145.3 (C-2'), 148.1 (C-5), 152.5 (C-8a), 157.5 (C-7), 161.2 (C-2).

Oxypeucedanin hydrate (**4**) - white crystals (6 mg); ^{1}H-NMR ($CDCl_3$ and CD_3OD, 500 MHz): δ 1.28, 1.31 (6H, s, 2 × Me), 3.86 (1H, dd, H-2″), 4.39 (1H, dd, H-1″a), 4.59 (1H, dd, H-1″b), 6.30 (1H, d, H-3), 7.04 (1H, d, H-3'), 7.16 (1H, s, H-8), 7.63 (1H, d, H-2'), 8.32 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$ and CD_3OD, 125 MHz): δ 24.9, 25.8 (2Me), 71.4 (C-3″), 74.3 (C-1″), 76.5 (C-2″), 94.1 (C-8), 105.0 (C-3'), 106.9 (C-4a), 112.3 (C-3), 114.0 (C-6), 139.6 (C-4), 145.1 (C-2'), 148.8 (C-5), 152.3 (C-8a), 158.1 (C-7), 161.5 (C-2).

Imperatorin (**5**) - white crystals (4.5mg); ^{1}H-NMR ($CDCl_3$ and CD_3OD, 500 MHz): δ 1.67, 1.71 (6H, s, 2 × Me), 4.09 (1H, m, H-2″), 4.50 (1H, m, H-1″a), 4.93 (1H, m, 1″b), 6.40 (1H, d, H-3), 6.85 (1H, d, H-3'), 7.44 (1H, d, H-5), 7.75 (1H, d, H-2'), 7.87 (1H, d, H-4); ^{13}C-NMR ($CDCl_3$ and CD_3OD, 125 MHz): δ 27.9, 29.6 (2Me), 70.5 (C-3″), 75.4 (C-1″), 76.8 (C-2″), 106.7 (C-3'), 113.4 (C-5), 114.1 (C-3), 116.3 (C-4a), 126.4 (C-6), 131.5 (C-8), 142.9 (C-8a), 146.0 (C-4), 146.9 (C-2'), 148.4 (C-7), 161.3 (C-2).

Ferulic acid (**6**) - white amorphous powder (4 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.39 (1H, d, H-2'), 6.81 (1H, d, H-6), 7.14 (1H, dd, H-5), 7.34 (1H, d, H-3), 7.60 (1H, d, H-1').

Falcarindiol (**7**) - light brown oil (400 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.88 (3H, t, H-17), 1.27 (8H, overlap, H-12 to H-16), 2.10 (2H, m, H-11), 4.93 (2H, d, H-1), 5.20 (1H, d, H-3), 5.26 (1H, d, H-8), 5.51 (1H, m, H-2), 5.61 (1H, m, H-10), 5.92 (1H, m, H-9); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.0 (C-17), 22.5 (C-16), 26.5 (C-11), 28.05 (C-13), 28.1(C-14), 29.2 (C-15), 31.7 (C-12), 58.5 (C-8), 63.4 (C-3), 68.0 (C-5), 73.2 (C-6), 78.2 (C-4), 78.8 (C-7), 117.2 (C-1), 127.4 (C-10), 134.4 (C-9), 135.0 (C-2).

Conclusion

Seven compounds have been isolated from the roots of *L. officinale*, with five of these compounds reported from *L. officinale* for the first time. One of the coumarins, namely oxypeucedanin (**3**) had high activity against MDR *M. tuberculosis* compared to the other coumarins. Overall, the anti-MDR-TB activity of the ethyl acetate extract of roots of *L. officinale* can be attributed primarily to the polyacetylene falcarindiol (**7**) and the coumarin oxypeucedanin (**3**). Furthermore, the binding affinity and docking score of both of these compounds were comparable to the anti-TB drug isoniazid. Thus, on the basis of the molecular docking study, oxypeucedanin (**3**) and falcarindiol (**7**) may be proposed as potential inhibitors of 2-trans-enoyl-ACP reductase (InhA), a key enzyme involved in the biosynthesis of the mycobacterial cell wall.

Conflict of Interest

The authors declare they have no conflict of interest.

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