

Study on The Effect and Mechanism of Luteolin Against *Mycoplasma pneumoniae**

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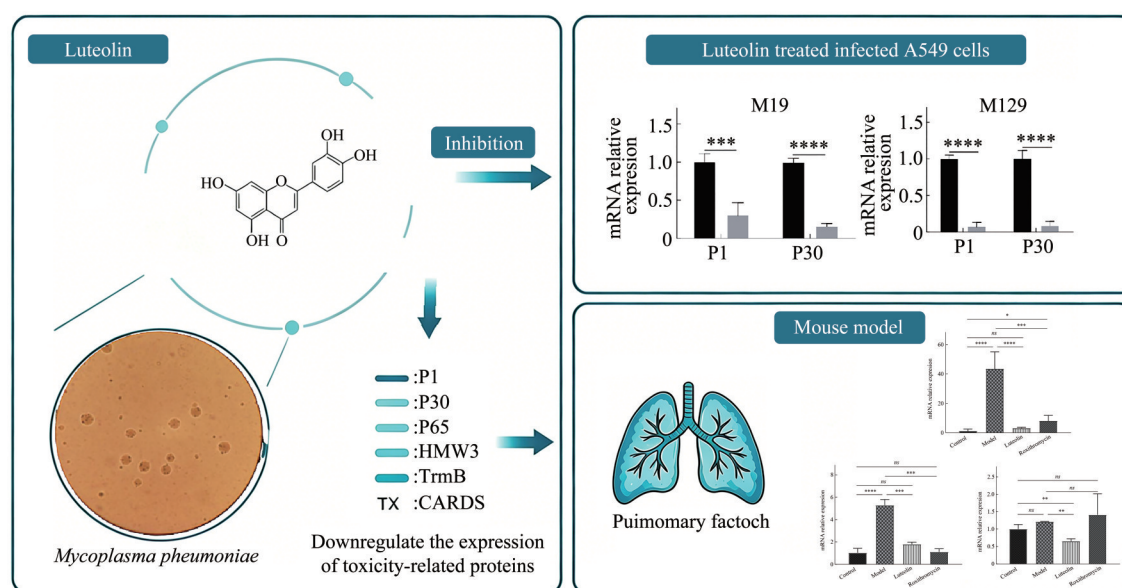
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Graphical abstract



Abstract Objective This study aimed to investigate the anti-*Mycoplasma pneumoniae* (MP) activity of luteolin and elucidate its underlying mechanisms. **Methods** Luteolin was identified as the primary active compound from the polyphenol extract of *F. diotrys* using network pharmacology. Its efficacy was evaluated against two MP strains: the standard strain M129 and the multidrug-resistant strain M19. A modified culture medium with visual characteristics was employed to determine the minimum inhibitory concentration (MIC) of luteolin. The expression of key proteins involved in MP growth and pathogenicity was assessed by

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qRT-PCR following luteolin treatment. Additionally, the viability of A549 cells infected with MP was compared between luteolin-treated and untreated groups. *In vivo* anti-MP activity was evaluated using a mouse model, and the expression of inflammatory cytokines in lung tissues was analyzed. **Results** Luteolin effectively inhibited both MP strains, with MIC_{90} values of 100 mg/L for M19 and M129. Treatment with luteolin significantly downregulated the expression of adhesion proteins P1 and P30 in both strains. However, the expression of P65, HMW3, TrmB, and CARDS TX was reduced only in the M19 strain following luteolin intervention. Luteolin also enhanced the growth and viability of A549 cells infected with MP. In the mouse model, luteolin treatment resulted in steady weight gain and was well tolerated. The bacteriostatic rate of luteolin in lung tissues was 50.7%, significantly higher than the 25.2% observed in the roxithromycin group. Furthermore, luteolin reduced the expression of inflammatory factors, including IL-6, TNF- α , and HMGB1, in MP-infected mice. **Conclusion** Luteolin effectively and safely inhibits the proliferation and pathogenicity of MP, particularly the drug-resistant M19 strain, by downregulating the expression of toxicity-associated proteins (P1, P30, P65, HMW3, TrmB, CARDS TX) and modulating host inflammatory responses. These findings suggest that luteolin may offer a novel therapeutic strategy for treating MP infections, especially those caused by drug-resistant strains.

Key words *Mycoplasma pneumoniae*, luteolin, adhesive proteins, CARDS TX, TrmB

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Mycoplasma pneumoniae (MP) infection, a type of self-limiting disease, is the major pathogenic cause of community-acquired pneumonia and bronchitis. However, in some patients, especially children, the infection can lead to severe pneumonia, asthma, and can even be fatal^[1-2]. As a human pathogen, MP has a very small genome of 0.58–2.20 Mb, and could not even quadruple without cholesterol, yet it was a highly pleomorphic microorganism with a specialized tip organelle responsible for its cytoadherence^[3-4].

Critical to the pathogenesis of MP is its ability to adhere to host cells^[5-7]. MP can form adhesion organelles in the membrane. These organelles are composed of adhesion proteins, adhesion helper proteins and molecular chaperone proteins. They made it easier for MP to take up nutrients by inserting microtubules into the cells and releasing substances such as the community acquired respiratory distress syndrome toxin (CARDS TX)^[8-9]. This eventually led to injury, necrosis and shedding of host cells, resulting in pneumonia and even extrapulmonary complications.

MP lacks a cell wall, rendering it inherently resistant to all antimicrobial agents that target the cell wall; by contrast, it is sensitive to antibiotics acting on bacterial DNA replication or protein synthesis, including fluoroquinolones, macrolides (erythromycin, azithromycin, osamycin) and tetracyclines, which are thus applied in the early clinical treatment of MP pneumonia (MPP)^[10-11]. Among these agents, macrolides have become the first-line drugs for MPP due to their potent anti-MP activity and mild adverse effects. However,

tetracyclines were limited in clinical use because they could directly irritate the gastrointestinal tract to induce obvious intestinal reactions, and even cause hepatotoxicity and enamel hypoplasia in children; fluoroquinolones also had a restricted application scope as they might interfere with cartilage and bone development in children^[12-14]. Unfortunately, the abuse and misuse of macrolides had led to a global surge in the prevalence of macrolide-resistant MP (MRMP), with its incidence ranging from 13.6% to 100% across regions and reaching as high as 69%–95% in clinical MP cases in Japan and China^[13-15]. MRMP infections progress to severe and refractory pneumonia accompanied by various extrapulmonary complications such as erythema nodosum, hepatic impairment and central nervous system damage, and crucially, there is currently no effective treatment for such infections, posing great challenges to the control of MP infection and the management of MPP^[12, 15]. The mechanisms underlying MP resistance involved antibiotic target site mutation, ribosomal protein amino acid mutation, ribosomal RNA methylation modification, active antibiotic efflux and antibiotic inactivation^[16], among which target site mutation has been clearly identified as the primary mechanism of MRMP resistance. Specifically, nucleotide sequence alterations in the central loop of the 23S rRNA V region of the ribosome, such as the most common mutations at sites A2063G, A2064G and A2063C, reduce the binding affinity between macrolides and ribosomes, thereby impairing the inhibition of MP protein peptide chain elongation and ultimately

leading to drug resistance^[17]. Therefore, the development of novel anti-resistant drugs is urgently required.

Traditional Chinese medicine (TCM) has a long history of treating MP infections^[18-19]. The way TCM treated MP was complex. It can inhibit MP adhesion and infection, inhibit apoptosis of airway epithelial cells, restore barrier function, inhibit systemic inflammatory response, and adjust abnormal immune function after infection^[20]. In our previous study, we found that *Fagopyrum dibotrys* (*F. dibotrys*), a traditional Chinese medicine usually used to treat lung diseases and throat inflammation, had a good effect against MP, especially MRMP.

Luteolin showed antibacterial activity against a wide range of bacteria, including both Gram-positive and Gram-negative strains. *In vitro* studies have found that it can inhibit the growth of *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Trueperella pyogenes*. The antibacterial mechanisms mainly involve increasing bacterial membrane permeability, inhibiting the synthesis of essential enzymes, and interfering with bacterial metabolic pathways. It also inhibited the growth of *Trueperella pyogenes* by disrupting membrane integrity and inhibiting bacterial enzyme activity.

Luteolin has well documented anti-inflammatory effects, which are mainly achieved by regulating multiple inflammatory signaling pathways and inhibiting the production of inflammatory mediators. It can effectively suppress the expression of pro-inflammatory cytokines like tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), as well as inflammatory enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). The key signaling pathways targeted by luteolin include nuclear factor- κ B (NF- κ B), mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription 3 (STAT3). By inhibiting the activation of these pathways, luteolin blocked the transcription and translation of inflammatory-related genes, thereby reducing the inflammatory response. *In vivo* studies have confirmed that luteolin can alleviate inflammatory reactions in various disease models, such as carrageenan-induced paw edema in mice, and have shown therapeutic potential in inflammatory lung diseases like chronic obstructive pulmonary

disease (COPD) and acute lung injury (ALI). Its anti-inflammatory properties are crucial for treating MPP, as MP infection often triggers excessive inflammatory responses that cause lung tissue damage and extrapulmonary complications.

So, luteolin has a broad spectrum of antibacterial and potent anti-inflammatory activities, and its multi target mechanism and synergistic effect with antibiotics make it a promising candidate for treating MP infections, especially MRMP strains. This study innovatively explored the application of luteolin in anti-MRMP, provided a new method for solving the clinical problem of MRMP drug resistance, and laid a foundation for the development of new anti-resistant drugs and combination therapy strategies. Future research should focus on *in vivo* efficacy evaluation, clinical trials, and the optimization of delivery systems to promote the clinical application of luteolin in the treatment of MPP.

In order to explore the reason for *F. dibotrys* anti-MP, the active monomer luteolin was screened from the polyphenol extract of *F. dibotrys* through network pharmacology firstly, and then was used to treat the MRMP strain M19 and the standard strain M129 respectively *in vitro*. Further, luteolin was used to treat the M19 model mice to evaluate the anti-MP effect and the mechanism of luteolin *in vivo*. It was found that luteolin had a good anti-MP effect, especially against the MRMP strain M19. Luteolin could inhibit MP's replication and decrease MP's toxicity by reducing the expression of P1, P30, P65, HMW3, TrmB and CARDS TX in MP and inhibit the systemic inflammatory response in MP model mice after being treated by luteolin. This study provides a theoretical basis for the development of anti-MRMP drugs, especially for drug-resistant MP.

1 Materials and methods

1.1 Materials

M19 is a type II strain of MP that was resistant to roxithromycin (macrolides) preserved in our laboratory; MP29342 was a type I standard strain purchased from ATCC. All details of the used MP strains in this study are listed in Table 1.

The A549 cells were obtained from the Institute of Medical Biology, Chinese Academy of Medical Sciences; luteolin was purchased from MedChemExpress LLC; BALB/c mice were

Table 1 The used *Mycoplasma pneumoniae* strains in this study

Type	Strain	Abbreviation	Characteristics
I	ATCC 29342	M129	Standard strain
II	wild strains	M19	MDR
II	ATCC 15531	M155	Standard strain

purchased from the Animal Laboratory Center of the Institute of Medical Biology.

1.2 Methods

1.2.1 MP culturing

The modified medium (PPLO medium 21 g; yeast powder 5 g; and sterile glucose 10 g) was added to ultrapure water. Then, 10 ml 0.2% phenol red was added to the medium to ensure that the volume was 800 ml and adjust the pH to 7.8. Finally, 200 ml sterile horse serum was added. Once all the liquids were mixed, they were aliquoted to the required volume. MP was passed at a ratio of 1 : 10 when the medium changed colour from red to yellow. After culturing at 37°C for 2 weeks, the color change units (CCU) and colony forming units (CFU) were calculated. CFU was calculated by the following formula: mean number of clones/dilution ratio/0.1.

1.2.2 Network pharmacology-based screening

In the present study, a network pharmacology strategy was employed to systematically investigate the potential therapeutic mechanisms of TCM components in the treatment of MP infection. Specifically, the chemical constituents of luteolin and the active monomers in the polyphenolic extracts of *F. diotrys* were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, 2020 edition). In accordance with the consensus on TCM network pharmacology research, potential active components were filtered using the established thresholds of oral bioavailability (*OB*) $\geq 30\%$ and drug-likeness (*DL*) ≥ 0.18 . The targets of the screened active components were predicted by submitting these components to multiple authoritative online databases and tools, including SwissTargetPrediction (Version 2), BATMAN-TCM (v2.0), Pharmacopoeia Database, SEA Database, and TCMSP Database. A threshold for target prediction probability ≥ 0.7 was set to ensure the reliability of the predicted results, and valid human target gene names were extracted accordingly. Concurrently, MP-related targets were retrieved from

GeneCards (v5.20), DisGeNET (v7.0), DrugBank Database, and OMIM Database with a gene-disease association score ≥ 0.3 . The retrieved targets from different databases were integrated through merging and deduplication processes to generate a standardized MP-related disease target set. Subsequently, Venn diagram analysis was conducted to identify the intersection targets between the active component target set and the MP-related disease target set. Cytoscape software (Version 3.8.0) was further utilized to construct the “active monomer-intersection target” interaction network, and the number of node connections (*i.e.*, degree value) was used as an indicator to assess the interaction intensity between active monomers and intersection targets. Notably, the integration of multiple databases for target prediction and disease-related target acquisition was designed to enhance the coverage and accuracy of target identification, while the screening of intersection targets and the construction of interaction networks provided a critical foundation for establishing the “component-target-disease” regulatory axis.

1.2.3 *MIC*₉₀ testing

The associated monomer activity was then verified by determining the *MIC*₉₀ (minimum inhibitory concentration for 90% MP). The monomeric drugs were first dissolved in DMSO and then diluted with medium in various ratios. According to the 1 : 1 volume ratio, the diluted monomeric drugs were added to the medium containing MP diluted 10 times with 10¹⁰ proportions continuously in a 96-well plate. The culture method was as described above and the color change of the medium was observed after 3 d. If the medium color was still red, the corresponding concentration of the monomeric drug was the *MIC*₉₀ value. The lower the *MIC*₉₀, the better the anti-MP effect. If the *MIC*₉₀ was greater than 100 mg/L, the anti-MP effect was considered poor.

1.2.4 MTT assay

A549 cells at the logarithmic growth stage were seeded in 96-well plates at a concentration of 5×10^3 /well and cultured at 37°C with 5% CO₂ from 24 to 48 h. Luteolin and roxithromycin were then serially diluted 10-fold each. Subsequently, 20 μ l each of these drug dilutions were added to the A549 cell medium per well. Seventy-two hours later, the MTT

assay kit was used to detect the cytotoxicity of luteolin and other drugs according to the value of UV light absorption at 490 nm.

1.2.5 Immunofluorescence assay

To determine whether MP can affect A549 cells, an immunofluorescence assay was performed. A549 cells were seeded in 6-well plates at 5×10^5 /well. Forty-eight hours later, the cells were washed three times and all liquid was discarded, then 4 ml MP suspension at 10^5 PFU/ml per well was added. After incubation for 4 h at 37°C, 4% formaldehyde was used to fix the cell bacteria co-cultures for immunofluorescence testing. The plate was then blocked with 2 000 µl 5% BSA for 2 h at room temperature. After washing the plate three times, mouse anti-MP antibody (diluted 1 : 300 in 2% BSA) was used as the primary antibody and incubated at 37°C for 1 h. Goat anti-mouse antibody (diluted 1 : 1 000 in 2% BSA) was used as the secondary antibody and incubated at 37°C for 1 h. Finally, 800 µl of DAPI solution (diluted 1 : 500 in PBS) was added and incubated for 20 min at room temperature in the dark. The immunofluorescence IF value was then observed and calculated under a fluorescence microscope.

1.2.6 RNA extraction and qRT-PCR assay

RNA from MP was extracted according to the instructions of the mycoplasma assay kit (Vazyme Biotech, Nanjing, D101-01/02). cDNA was obtained by reverse transcription according to the instructions of the reverse transcription kit (Yi Sheng, Nanjing, 11123ES60). The primers used for the real-time fluorescence quantitative PCR detection are listed in Table 2. The reaction process of PCR: 95°C 3 min; 95°C 15 s, 60°C 30 s, 40 cycles; 4°C 1 h.

1.2.7 Animal study

120 Female BALB/c mice (4–6 weeks old) were infected intranasally with 50 µl of the drug-resistant strain M19 at a MP titer of 10^9 CCU/ml to obtain MP-infected mice. After infection with M19 for 5 d, the M19-infected mice were treated with luteolin at a dose of 50 mg/kg or ruxithromycin at a standard dose of 50 mg/kg by intragastric administration, respectively. The model group was given saline and M19. Mice without M19 infection were used as the control group.

The mice were weighed daily. After 12 d, euthanasia was performed and lung tissue was collected and weighed. The expression of

Table 2 The gene sequence of primers

Primers	Sequence (5'-3')
<i>P1</i> forward	CTTTGCGTTACGCCGGTATG
<i>P1</i> reverse	GGCGTTTGCGGGTTTAACTT
<i>P30</i> forward	CTGGTACTGGTTGTGGTGCT
<i>P30</i> reverse	CAACGCATTCTGCCCAACA
<i>CARDS TX</i> forward	ATGCTGTTCCCGTTGAACCT
<i>CARDS TX</i> reverse	GGTGGACTTCAATCAGGGCA
<i>TrmB</i> forward	GCGTGAACCCACGATTGTTT
<i>TrmB</i> reverse	ATGTGGAGCAGTTAGTCGCC
<i>P65</i> forward	AACGCCATCTTTTCGATGCG
<i>P65</i> reverse	ACGTTGCATGCGTTCCTCTA
<i>HWM3</i> forward	CCGACTGGCAATTGGTGTTG
<i>HWM3</i> reverse	TTTGGGTCACCTCCACGATG
<i>IL-6</i> forward	CAACGATGATGCACTTGCAGA
<i>IL-6</i> reverse	TGTGACTCCAGCTTATCTCTTG
<i>TNF-α</i> forward	CAGGCGGTGCCTATGTCTC
<i>TNF-α</i> reverse	CGATCACCCCGAAGTTCAGTAG
<i>HMGB1</i> forward	GCTGACAAGGCTCGTTATGAA
<i>HMGB1</i> reverse	CCTTTGATTTTGGGGCGGTA
<i>GAPDH</i> forward	GTCTTCACCACCATGGAGAAGGC
<i>GAPDH</i> reverse	TTGTTGTCATGGATGACCTTGGCC

inflammatory cytokines (IL-6, TNF-α, HMGB1 and IL-17A) in the lung tissues was measured by qRT-PCR. MP proliferation rate in lung tissues was determined by CFU counting.

This study was conducted in strict accordance with the Declaration of Helsinki and the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of Kunming University of Science and Technology (approval number: KUST-2019-11). All surgeries were performed under anaesthesia and great care was taken to minimize suffering.

1.2.8 Detection of adhesion protein and toxicity protein expression levels by Western blot (WB)

By detecting the protein-level expression of adhesion proteins and toxicity proteins, the interventional effect of luteolin on MP could be further verified, which contributes to the analysis of its therapeutic mechanism. Samples were collected sequentially as follows: cultures of M19 in modified medium, cultures of A549 cells infected with M19, bronchial tissues of mice infected with M19, cultures of A549 cells infected with M19 and intervened with

luteolin, bronchial tissues of M19-infected mice treated with luteolin, and bronchial tissues of healthy control mice. Primary antibodies included rabbit anti-P30 and rabbit anti-CARDS, respectively, which were diluted 2 000-fold in PBS. The secondary antibody was a goat polyclonal antibody against MP, diluted 5 000-fold in PBS. All antibodies used in this experiment were purchased from the Institute of Medical Biology, Chinese Academy of Medical Sciences.

1.3 Statistical analysis

IBM® SPSS® Statistics version 21.0 was used for statistical analysis. One-way analysis of variance was used to test for differences in protein expression between groups. mRNA expression was expressed as mean±SD from three or more independent experiments. $P<0.05$ was used to define statistical

significance.

2 Results

2.1 The modified medium was suitable for the culture of MP

A visualized culture system was constructed that was conducive to propagating MP. It is suitable for separating and culturing MP. Two MP strains, each with its own biological characteristics, were cultured in the modified medium. After 3 d, all MP strains were detected by CCU and CFU methods. It was found that all concentrations of MP reached 1.33×10^7 CFU/ml (Figure 1). These results indicated that the modified culture system was beneficial for the proliferation of MP and provided basic culture conditions for the study of anti-MP.

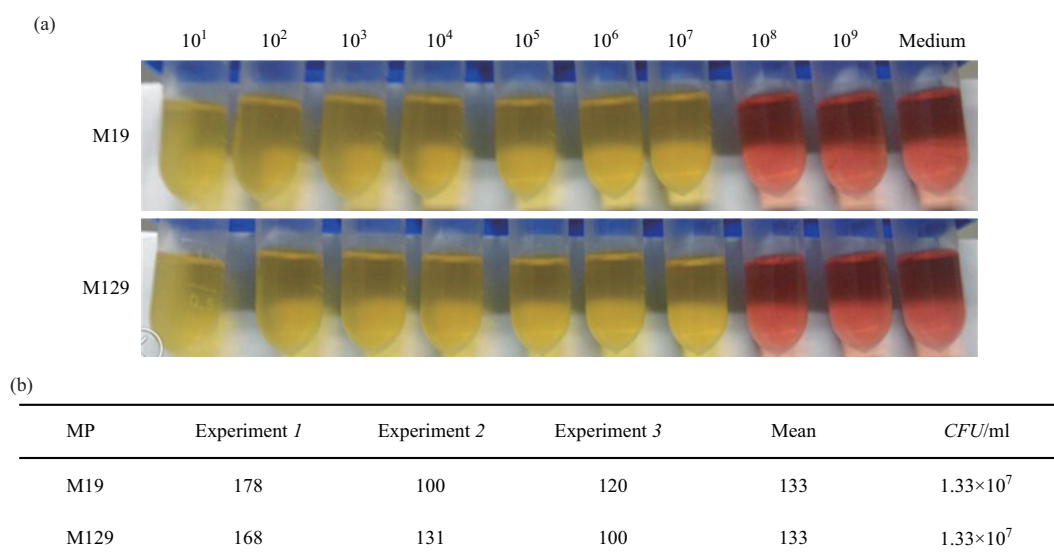


Fig. 1 The concentrations of M19 and M129 were determined

(a) CCU method; (b) CFU method.

2.2 Luteolin is the most effective ingredient

According to $OB>30\%$ and $DL>0.18$, 15 monomers in polyphenolic extracts of *F. diotrys* were obtained from the TCMSP database, including quercetin, luteolin, emodin, etc. (Table 3).

Database analysis yielded 599 potential targets for active monomers and 242 targets for MP treatment, respectively, and Venn diagram analysis revealed that there were 70 intersectional targets between them (Figure 2a). This set of intersectional targets represented the primary focus for the

development of novel anti-MP agents. The “active monomers-intersectional targets” interaction network showed that quercetin and luteolin had the highest degree of correlation with the intersectional targets (Figure 2b).

Then, the activity of 6 monomers including luteolin, quercetin, catechin, flavanone, 3,4-dihydroxybenzoic acid and isorhamnetin were each tested. The results showed that only the MIC_{90} value of luteolin was 100 mg/L, and the MIC_{90} value of other monomers was greater than 100 mg/L against

Table 3 15 active monomers in polyphenol extracts of <i>F. diotrys</i>				
Mol ID	Molecule name	MW	OB/%	DL
MOL000098	Quercetin	302.25	46.43	0.28
MOL002914	Eriodyctiol (flavanone)	288.27	41.35	0.24
MOL000354	Isorhamnetin	316.28	49.6	0.31
MOL000358	beta-sitosterol	414.79	36.91	0.75
MOL000359	Sitosterol	414.79	36.91	0.75
MOL000004	Procyanidin B1	578.56	67.87	0.66
MOL000492	(+)-catechin	290.29	54.83	0.24
MOL000569	Digallate	322.24	61.85	0.26
MOL000006	luteolin	286.25	36.16	0.25
MOL000631	Coumaroyltyramine	283.35	112.9	0.2
MOL006504	(-)-catechin gallate	442.4	53.57	0.75
MOL007280	3-methylquercetin	316.28	30.85	0.3
MOL000073	ent-Epicatechin	290.29	48.96	0.24
MOL007301	3,4-Dihydroxybenzoic acid	312.29	59.7	0.49
MOL000096	(-)-catechin	290.29	49.68	0.24

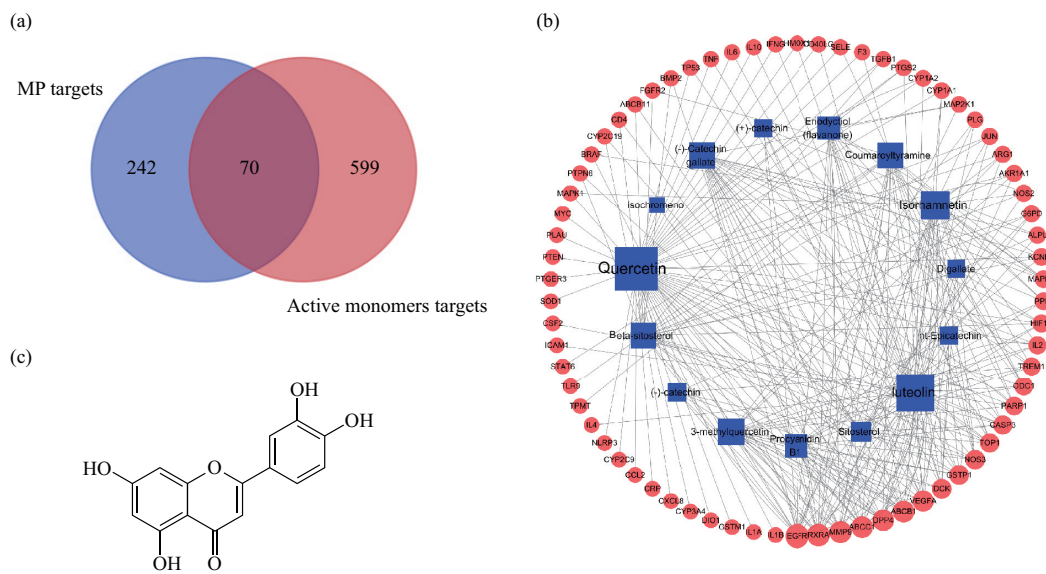


Fig. 2 Screening the active monomers from polyphenol extracts of *F. diotrys* through network pharmacology (a) Venn diagram; (b) the active monomers-intersection targets interaction network; (c) luteolin chemical structure.

the two strains of MP (Table 4). These results suggested that luteolin was the most effective active monomer against MP in the polyphenol extract of *F. diotrys*. The chemical structure of luteolin is shown in Figure 2c.

2.3 Luteolin inhibited the expression of adhesive proteins P1 and P30

Adhesion of MP to airway epithelial cells is the key step in its pathogenic process, and the adhesion proteins P1 and P30 play a crucial role^[21-22]. To investigate the effect of luteolin treatment on the expression of P1 and P30, MP was first treated

Table 4 The MIC₉₀ values of 6 active monomers which had the highest degree of correlation with the intersectional targets for M19 and M129 respectively

Monomers	MIC ₉₀	
	M19	M129
Luteolin	100 mg/L	100 mg/L
Catechin	125 mg/L	125 mg/L
Flavanone	250 mg/L	250 mg/L
Quercetin	>100 mg/L	>100 mg/L
3,4-Dihydroxybenzoic acid	>100 mg/L	>100 mg/L
Isorhamnetin	>100 mg/L	>100 mg/L
Roxithromycin	200 μmol/L	1 μmol/L

directly with luteolin. The mRNA of each MP strain was then detected using a mycoplasma assay kit, and cDNA was obtained using a reverse transcription assay kit. Then, qRT-PCR was performed to detect the expression of P1 and P30 of MP, and the results showed that the expression of P1 and P30 in MP19 and M129 strains were significantly decreased after direct treatment with luteolin (Figure 3a).

We tested the ability of MP to infect A549 cells by immunofluorescence assay to investigate the effect of luteolin treatment on the expression of P1 and P30 after MP infection. A549 cells were infected with MP for 4 h, followed by incubation with luteolin for 48 h. Rabbit anti-MP was used as the primary antibody, and

goat anti-rabbit IgG (labelled with FITC) was used as the secondary antibody. The results showed that MP was distributed and accumulated in large numbers around the nucleus, suggesting that the two MP strains could effectively infect A549 cells (Figure 3b).

To detect the effect of luteolin on the expression of P1 and P30 in MP-infected A549 cells, A549 cells were infected with M19 and M129 for 4 h and then treated with luteolin for 48 h. qRT-PCR was performed as described above. The results showed that luteolin also had an inhibitory effect on the expression of P1 and P30. The effect of luteolin was more significant than that of the direct treatment with MP (Figure 3c).

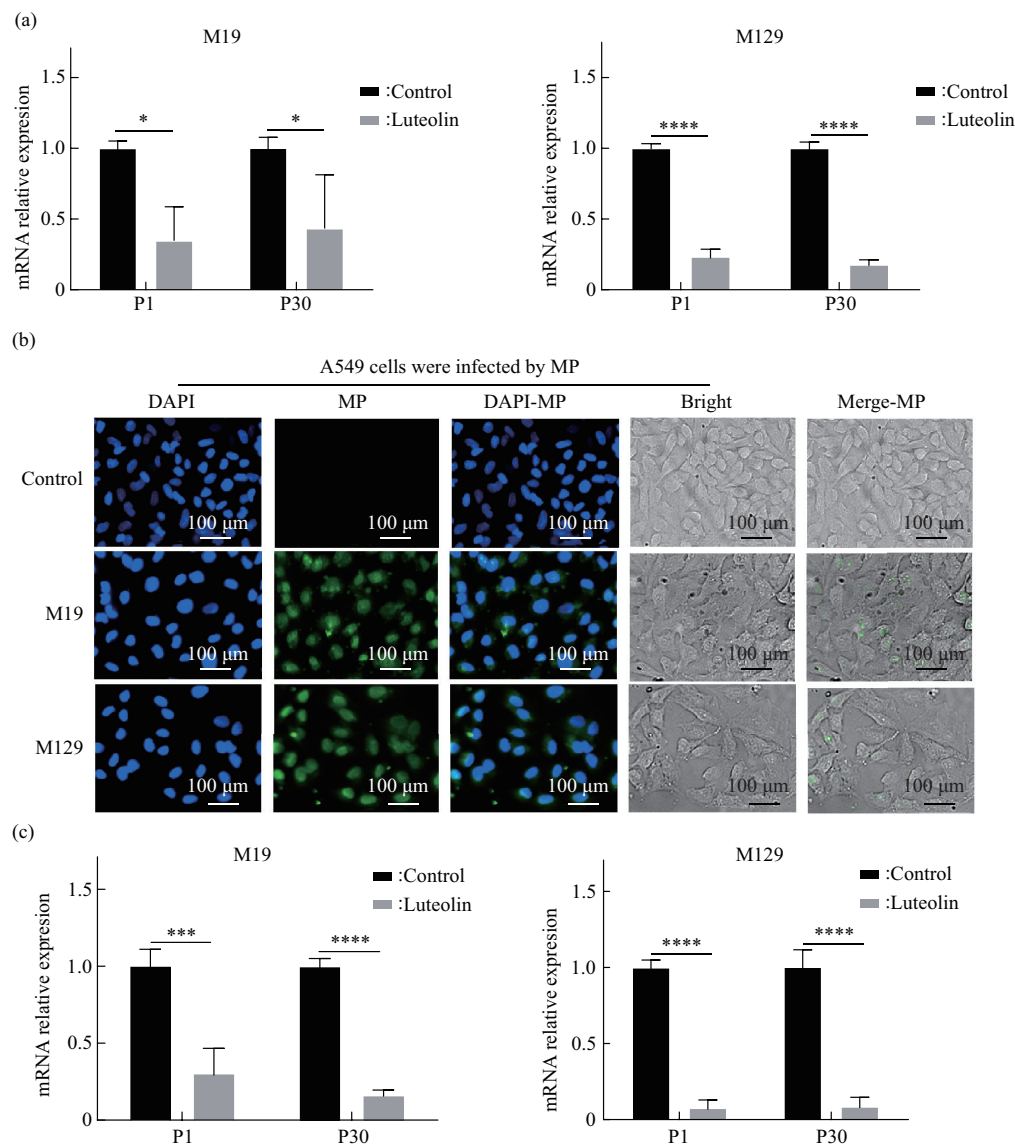


Fig. 3 The effect of luteolin on the expression of adhesion proteins P1 and P30

(a) The expression of adhesion proteins in M19 and M129 treated with luteolin directly. (b) The infection of M19 and M129 in A549 cells was detected by immunofluorescence assay. (c) The expression of adhesion proteins in M19 and M129 treated with luteolin after infecting A549 cells.

* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

2.4 Luteolin has the adhesion inhibition effect

The standard reference strain of MP, strain M155, was used for the adhesion inhibition assay. A549 cells were seeded in 6-well plates 24 h in advance at a density of $1 \times 10^6/\text{ml}$. Subsequently, M155 strain was diluted with PBS to a concentration of 10^5 PFU/L and mixed with luteolin (final concentration: 100 mg/L) at an equal volume. All culture medium from the A549 cell wells was discarded, and the cell monolayers were washed three

times with PBS. The mixture was then added to the wells, followed by co-incubation with A549 cells at 37°C for 6 h. After co-incubation, the cell monolayers were washed three times with PBS again, and an immunofluorescence assay was performed to detect MP, so as to verify the direct inhibitory effect of luteolin on MP adhesion. The results demonstrated that luteolin effectively blocked the adhesion of the standard reference strain M155 to A549 cells, with a significant adhesion inhibition effect (Figure 4).

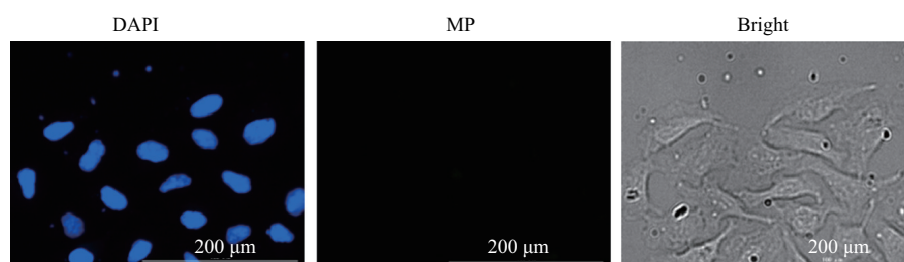


Fig. 4 Observation of adhesion inhibition assay

2.5 Luteolin reduced the expression of adhesion helper proteins P65 and HMW3

In order to investigate the effect of luteolin on adhesion helper proteins, the mRNA expression levels of *P65* and *HMW3* were measured by qRT-PCR after M19 and M129 strains were treated with luteolin and roxithromycin, respectively, for 48 h as described above. The results showed that when MP was treated directly with luteolin, the mRNA expression levels of *P65* and *HMW3* were slightly up-regulated in the M19 strain (Figure 5a). However, when MP was cocultured with A549 cells and then treated in the same way with luteolin, the mRNA expression levels of *P65* and *HMW3* were significantly decreased. This decreasing trend was more evident for M19 than for M129 (Figure 5b). The same trend was observed for the intervention effect of roxithromycin (Figure 5c). The results indicated that luteolin had the same inhibitory effect as roxithromycin on the expression of *P65* and *HMW3* proteins, in M19 strain but not M129.

2.6 Luteolin downregulated the expression of TrmB

The tRNA (guanine-N(7))-methyltransferase (TrmB) is a tRNA methyltransferase that participates in the biosynthesis of N(7)-methylguanine-tRNA and is essential for tRNA transport^[23-24]. If the expression of TrmB decreased, the tRNA would be disorder

structure and without function, even the process of protein synthesis would be disorder, ultimately, MP proliferation was affected.

To investigate the effect of luteolin on the expression of TrmB in MP, M19 was treated with luteolin and roxithromycin. The results showed that luteolin significantly reduced the expression of TrmB in M19 group, but not in M129 (Figure 6). It could be concluded that luteolin exerted differential intervention effects on different MP strains. For type II strains (*e.g.*, MP19 in this study), luteolin could significantly inhibit the expression of TrmB. Notably, this inhibitory effect remained pronounced even when the strains developed resistance to roxithromycin. However, it was also found that the inhibitory effect of luteolin on TrmB expression in type I MP strains (*e.g.*, M129) was weaker than that of roxithromycin.

2.7 Luteolin reduced the toxicity by blocking CARDS TX

CARDS TX was a unique cytotoxin from MP that could induce ADP ribosylation and cell vacuolization. If CARDS TX were present in the airways, it would efficiently bind to surfactant protein A1 and membrane protein A2 in airway epithelial cells. The protein could then be internalised into airway cells, resulting in cell vacuolization and

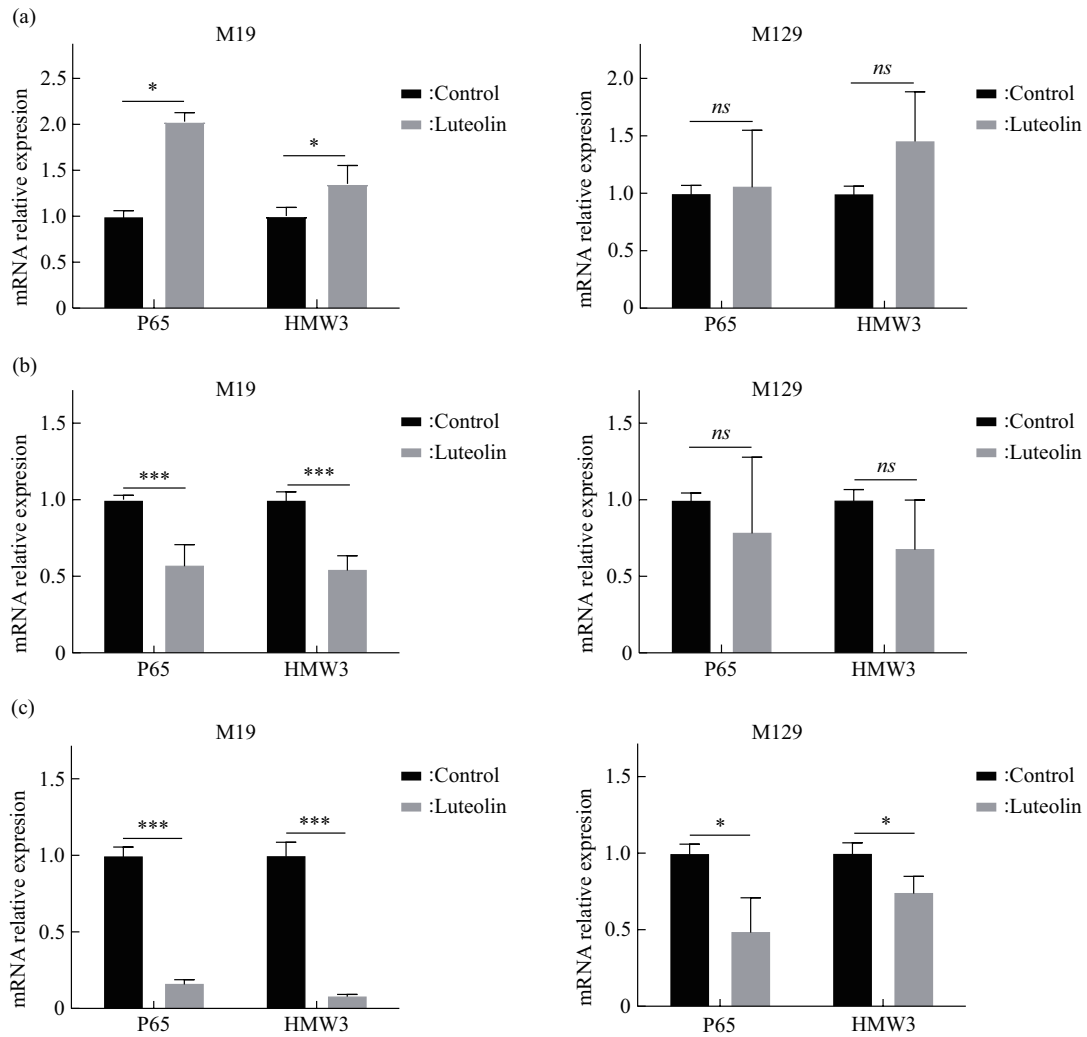


Fig. 5 The effect of luteolin on the expression of adhesion helper proteins P65 and HMW3

(a) The expression of adhesion helper proteins in M19 and M129 treated by luteolin directly. (b) The expression of adhesion helper proteins in M19 and M129 treated with luteolin after infecting A549 cells. (c) The expression of adhesion helper proteins in M19 and M129 treated with roxithromycin after infecting A549 cells. * $P < 0.05$, *** $P < 0.001$, ns, not significant.

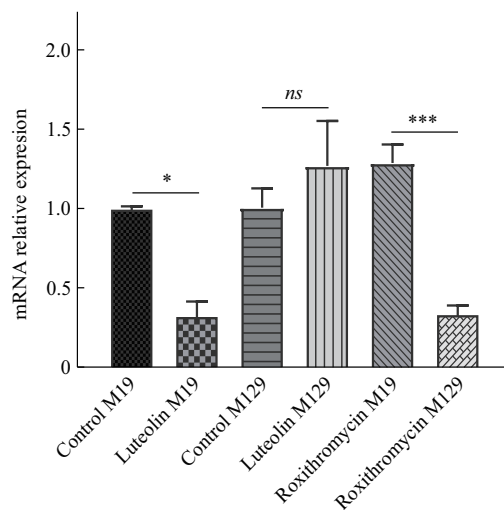


Fig. 6 The effect of luteolin on the expression of TrmB in MP

* $P < 0.05$, *** $P < 0.001$, ns, not significant.

impaired ciliary movement, leading to cell death and a range of extrapulmonary complications^[8, 25-26].

Earlier studies demonstrated up-regulation of *CARDS TX* expression during MP infection. As described above, M19 and M129 strains were treated with luteolin and roxithromycin, respectively. The expression of *CARDS TX* was measured by qRT-PCR after 48 h of luteolin treatment. The expression of *CARDS TX* decreased after treatment in the M19 group but not M129 group. The drug resistant strain M19 showed the same trend as described above (Figure 7a).

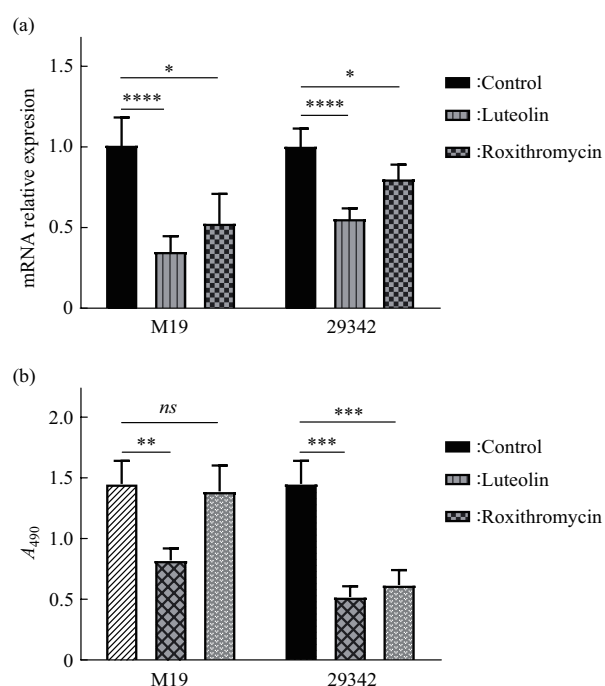


Fig. 7 The effect of luteolin on the toxicity of MP

(a) The effect of luteolin on the expression of toxic protein *CARDS TX* in MP. (b) The viability recovery of A549 cells infected by MP after being treated with luteolin. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns, not significant.

Meanwhile, the viability of A549 cells after luteolin treatment was determined by MTT assay. Both strains M19 and M129 had strong toxicity to A549 cells; however, luteolin could significantly inhibit this cytotoxicity in the drug-resistant strain M19 (Figure 7b) as type I, but not strain MPM129 as type II. These results suggest that luteolin might reduce the cytotoxicity of M19 on MP-infected A549 cells by inhibiting the expression of *CARDS TX*.

2.8 Luteolin inhibited the proliferation of MP *in vivo*

To demonstrate that luteolin could inhibit MP

proliferation *in vivo*, 120 female BALB/c mice aged 4–6 weeks were infected with M19. Each mouse was given 50 μ l of MP suspension at a dose of 10^9 CCU/ml by nasal injection (*n. i.*). Five days later, the mice in the luteolin treatment group were administered luteolin (at a dose of 50 mg/kg, 1 ml) by intragastric injection (*i. g.*); the mice in the model group were treated with saline; the mice in the roxithromycin treatment group were treated with roxithromycin (50 mg/kg, 1 ml); while the mice not infected with M19 were used as control group. The weights of all mice were monitored daily for 12 d. The results showed that luteolin could help the animals to have a good condition and a trend of gradual recovery after administration (Figure 8a).

After isolation and identification of MP from these animal tissues and organs, MP loading was evaluated by calculating *CFU*. luteolin was able to inhibit the proliferation of M19 in the mouse lung. The M19 loading in the lungs of the luteolin treatment group was significantly lower than that of the model group (Figure 8b). It was worth noting that the bacteriostatic rate of luteolin was 44.9%, while that of roxithromycin was 25.2% for M19. The lung wet weights in the luteolin and roxithromycin treatment groups were almost the same as those in the control group, while the lung wet weights in the model group were significantly lower than those in the control group (Figure 8c). The results suggested that luteolin can effectively inhibit the proliferation of MP in animal model, and for M19, the inhibitory effect of luteolin was even more obvious than that of roxithromycin.

2.9 Luteolin reduces systemic inflammatory response *in vivo*

After MP infected host cells, Toll-like receptors could induce macrophages to release inflammatory cytokines, leading to the over-expression of immune factors that ultimately result in pneumonia^[27-28]. Among these, IL-6 was an important cytokine in the MPP inflammatory response, and its elevation was the earliest indicator of MPP infection^[29]. TNF- α is a multifunctional bioactive peptide produced mainly by monocytes and macrophages and involved in lung injury and severe pneumonia^[30-31]. High-mobility group protein 1 (HMGB1) is an active secretory cytokine that can be expressed by activated inflammatory cells and accumulates during MP

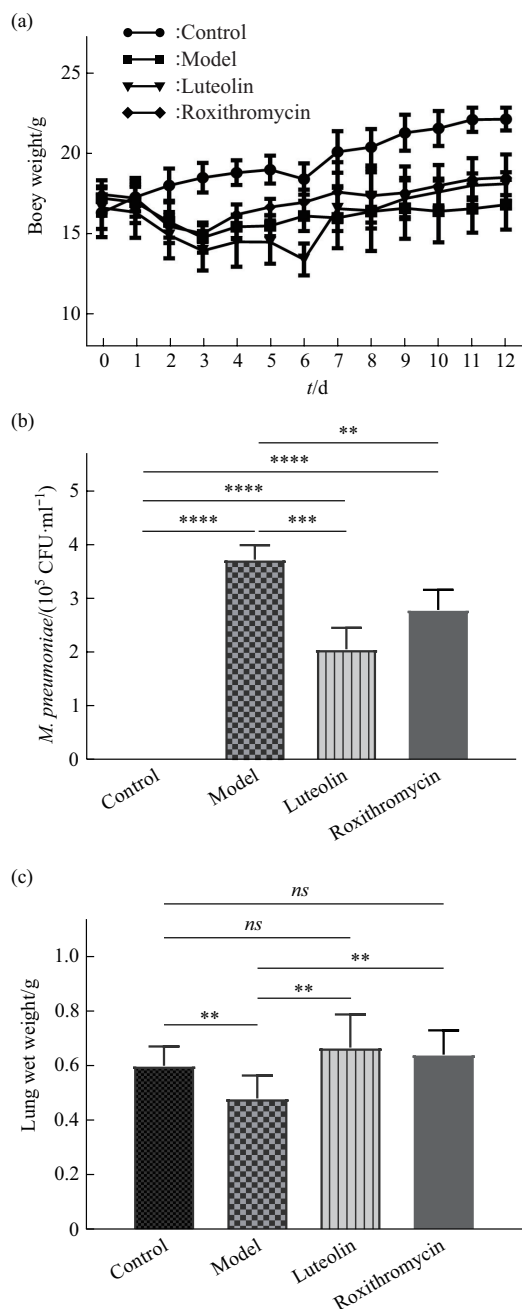


Fig. 8 Effects of luteolin on body weight, MP clearance and lung wet weight in mice

(a) The effect of luteolin on body weight; (b) the effect of luteolin on MP clearance in lung tissue by calculating CFU; (c) the effect of luteolin on lung wet weight. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, *ns*, not significant.

infection. Studies have shown that HMGB1 is associated with epithelial barrier disruption and organ dysfunction, and that HMGB1 expression was increased in children with refractory MP pneumonia compared to normal children^[32-33].

In our research, mice infected with M19 were treated with luteolin or roxithromycin. Normal mice

and model mice without drug treatment were used as controls. The expression of IL-6, TNF- α , HMGB1 in mouse lung tissues was measured by qRT-PCR. The results showed that the mRNA expression of IL-6 and TNF- α increased significantly in the model group, while HMGB1 increased slightly. After luteolin treatment, the mRNA expression of IL-6, TNF- α and HMGB1 decreased significantly (Figure 9). The results showed that luteolin had a better anti-inflammatory effect and significantly improved pneumonia caused by MP, especially the drug-resistant strain M19.

2.10 Luteolin interferes with adhesion and toxicity proteins expression

We specifically investigated the expression profiles of the adhesion protein P30 and the toxicity protein CARDS under the intervention of luteolin. As shown in Figure 10, high-level expression of the M19 adhesion protein P30 was detected in all the following samples: pure M19 cultures, cultures of A549 cells infected with M19, and bronchial tissues of M19-infected mice. However, no P30 protein expression was detected in the bronchial tissues of M19-infected mice after luteolin intervention.

The toxic protein CARDS is critical for the survival of infected mice. High-level expression of CARDS was observed in both the M19-infected A549 cell group and the M19-infected mouse group, indicating that CARDS plays a vital role in M19 infection and pathogenicity. Similarly, after luteolin intervention, no CARDS protein expression was detected either at the cellular level or in the bronchial tissues of the animals. CARDS was significantly inhibited by luteolin, thereby achieving the therapeutic effect. Furthermore, the CARDS-negative state is essential for the health of mice and can be used as an evaluation index for the cure of M19 infection after treatment.

3 Discussion

MP is the smallest known prokaryote capable of independent *in vitro* growth and is a causative agent of acute upper and lower respiratory tract infections as well as extrapulmonary complications^[34-35]. In children, MPP represents the most significant clinical manifestation of MP infection, accounting for over 30% of paediatric respiratory infections. MPP could induce multi-organ and multi-system damage and

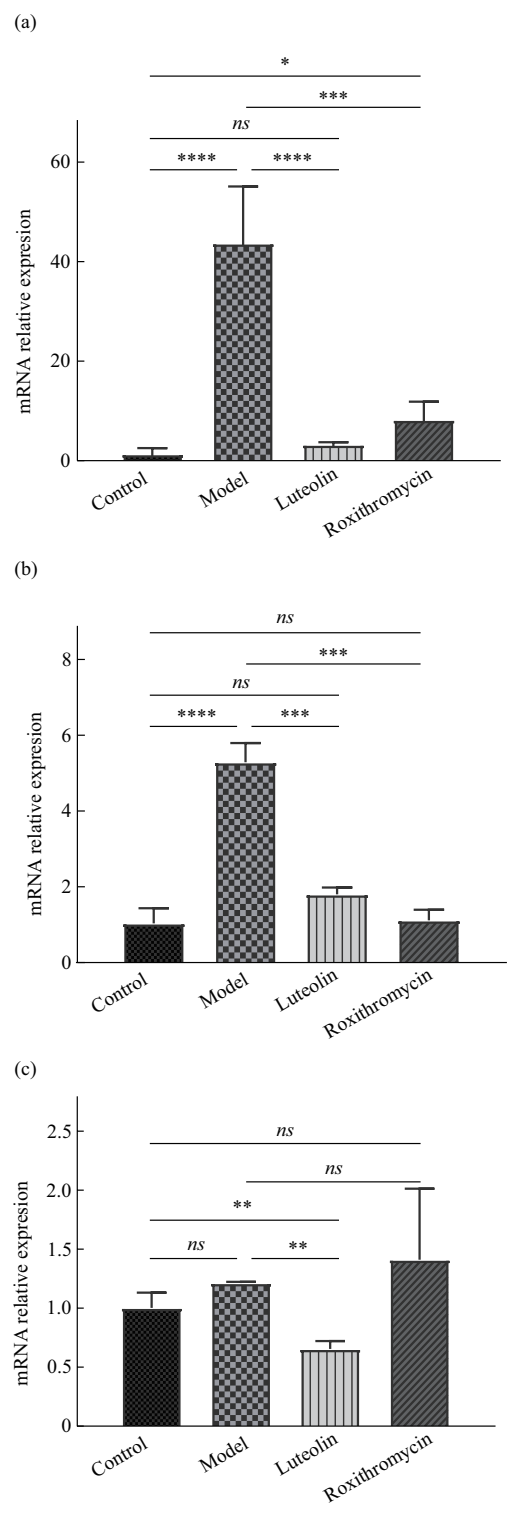


Fig. 9 The effect of luteolin on the expression of inflammatory factors

(a) The effect of luteolin on the expression of IL-6; (b) the effect of luteolin on the expression of TNF-α; (c) the effect of luteolin on the expression of HMGB1. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, ns, not significant.

dysfunction, and its associated morbidity and mortality have increased in recent years, posing a

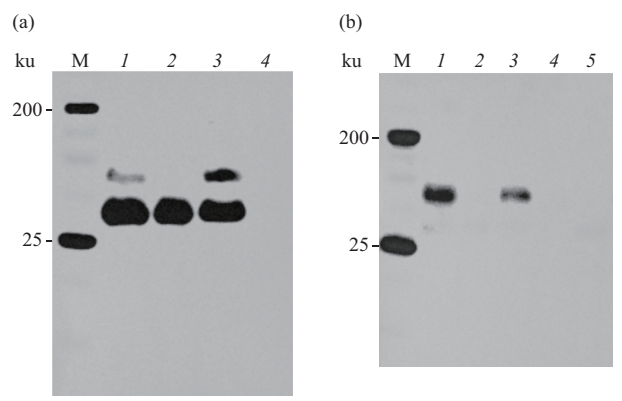


Fig. 10 Western blot assay of luteolin interfering with adhesion proteins and toxicity proteins of *Mycoplasma pneumoniae* strain M19

(a) Western blot assay of luteolin interfering with the adhesion protein P30 of M19. M: Marker; I: culture of A549 cells infected with M19; 2: culture of M19 in modified medium; 3: bronchial tissues of mice infected with M19; 4: bronchial tissues of M19-infected mice treated with luteolin. (b) Western blot assay of luteolin interfering with the toxicity protein CARDS in M19. M: Marker; I: culture of A549 cells infected with M19; 2: culture of A549 cells infected with M19 and intervened with luteolin; 3: bronchial tissues of mice infected with M19; 4: bronchial tissues of M19-infected mice treated with luteolin; 5: bronchial tissues of healthy control mice.

major threat to global public and child health^[36]. Our previous research demonstrated that polyphenol extracts from *F. diotrys*, a traditional Chinese medicine used for lung diseases and throat inflammation, possessed potent anti-MP activity, particularly against drug-resistant strains. Through network pharmacology screening, luteolin was identified as the principal active monomer. Luteolin is known for its broad pharmacological activities, including anti-inflammatory, anti-allergic, uric acid-lowering, anti-tumour, antibacterial, and antiviral effects^[37-38]. However, its efficacy against MP, especially drug-resistant MP, had not been previously reported. In this study, we evaluated the anti-MP activity of luteolin and explored its underlying mechanisms.

We found that luteolin exhibited significant anti-MP effects, with particularly potent activity against multi-drug-resistant strains (e.g., M19). Its inhibitory effect on the M19 strain was superior to that on standard strains (e.g., M129), with an MIC_{90} of only 100 mg/L as determined by CFU enumeration. To elucidate the mechanism, we examined the expression of several MP-related proteins. First, since MP infection requires adhesion to airway epithelial cells, a

process mediated by adhesion organelles comprising adhesion proteins (*e.g.*, P1, P30) and adhesion helper proteins (*e.g.*, P65, HMW3), we found that luteolin reduced the expression of P1 and P30 in both M19 and M129 strains, and decreased P65 and HMW3 expression in the M19 strain. Second, luteolin suppressed the expression of TrmB in the M19 strain, a key protein involved in MP mRNA transcription and translation, thereby disrupting tRNA function and limiting bacterial replication. Third, beyond inhibiting MP, luteolin promoted the growth of A549 cells and maintained high cellular viability by reducing the expression of the toxic protein CARDS TX, a distinct advantage over conventional treatments such as quinolones and tetracycline. Fourth, in animal studies, luteolin achieved a 44.9% clearance rate of MP from lung tissue, compared to 25.2% for roxithromycin. Luteolin also suppressed the overexpression of inflammatory factors *in vivo*, mitigating inflammation and protecting host tissues. These results indicated that luteolin was both safe and effective in animal models, suggesting potential clinical benefits. Its use could reduce reliance on glucocorticoids and antibiotics, offering a dual advantage for patients. However, further work was needed to detail the mechanism of luteolin against drug-resistant MP, including the role of MP resistance mechanisms.

Research on the anti-MP effects of luteolin remains exploratory, and critical gaps persist in understanding its mechanistic logic. While existing studies, including ours, confirmed that luteolin downregulates MP adhesion proteins such as P1 and P30, the specific regulatory pathways and molecular targets remain poorly defined. At the molecular level, luteolin might act transcriptionally by interfering with MP-specific transcription factors binding to promoter regions of adhesion protein genes, consistent with our finding that luteolin inhibited TrmB expression. Alternatively, it might operate post-transcriptionally by modulating mRNA stability or translation efficiency. Notably, comprehensive studies elucidating these precise regulatory details were scarce, and the interplay between luteolin's effect on adhesion proteins and TrmB-mediated transcriptional networks in MP remained unclear.

Preliminary evidence indicates that luteolin inhibits the expression of MP toxicity factors such as adhesion proteins (P1, P30) and toxic proteins (CARDS TX), while also modulating host immune responses. Our previous animal experiments

confirmed that luteolin downregulates pro-inflammatory cytokines (*e.g.*, IL-6, TNF- α , HMGB1) in MP-infected mice, thereby attenuating inflammation. Due to experimental limitations, we have integrated analyses of relevant transcription factors and signaling pathways based on existing literature. A key unanswered question was how luteolin precisely regulates the expression of both MP-related proteins and host cytokines, whether through targeting specific transcription factors (*e.g.*, TrmB in MP or NF- κ B in host cells) or interfering with core signaling pathways (*e.g.*, MAPK) involved in MP pathogenicity and host immunity. Deeper exploration of these aspects will clarify luteolin's comprehensive anti-MP mechanism and strengthen the foundation for its clinical translation.

The study also has some limitations. It was unable to procure specific monoclonal antibodies for the target proteins. Consequently, protein-level assays could not be performed precisely. Future studies will incorporate these experiments to enhance comprehensiveness and rigor.

In conclusion, luteolin demonstrated promising anti-MP activity, potentially through multiple mechanisms against M19-like MP: inhibiting MP adhesion by downregulating adhesion and helper proteins; limiting MP replication by suppressing TrmB expression; reducing MP cytotoxicity *via* decreased CARDS TX expression to maintain host cell viability *in vitro* and *in vivo*; and attenuating systemic inflammation by modulating inflammatory cytokine expression *in vivo*. This study offers a novel therapeutic strategy for drug-resistant MP infection.

4 Conclusion

Luteolin exhibited potent inhibitory activity against MP strains M19 and M129, primarily by downregulating critical adhesion and virulence-associated proteins including P1, P30, P65, HMW3, TrmB and CARDS TX, especially in the drug-resistant M19 isolate. It enhanced the viability of MP-infected A549 cells, showed favorable biosafety in murine models, and achieved a higher pulmonary bacteriostatic rate than roxithromycin. Moreover, luteolin attenuated the production of pro-inflammatory mediators such as IL-6, TNF- α and HMGB1. Collectively, these findings demonstrated that luteolin effectively and safely suppressed MP

proliferation and pathogenicity *via* modulating virulence factor expression and host inflammatory responses, highlighting its promising potential as a novel therapeutic candidate against MP infection.

References

- [1] Ding L, Jiang Y. Biomarkers associated with the diagnosis and prognosis of *Mycoplasma pneumoniae* pneumonia in children: a review. *Front Cell Infect Microbiol*, 2025, **15**: 1552144
- [2] Miyashita N, Ogata M, Fukuda N, *et al.* Macrolide-resistant *Mycoplasma pneumoniae* infection prevalence increases again in Osaka. *Respir Investig*, 2025, **63**(4): 517-520
- [3] Tang Y, Fu X, Li X, *et al.* Multi-omics analysis of human plasma reveals reprogramming of tryptophan metabolism associated with inflammation in *Mycoplasma pneumoniae* pneumonia in children. *J Infect*, 2025, **91**(1): 106525
- [4] Yan C, Tong S, Wu Y, *et al.* Macrolide-resistant *Mycoplasma pneumoniae* resurgence in Chinese children in 2023: a longitudinal, cross-sectional, genomic epidemiology study. *Lancet Microbe*, 2025, **6**(12): 101200
- [5] Bolormaa E, Park J Y, Choe Y J, *et al.* Treatment of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children: a meta-analysis of macrolides versus tetracyclines. *Pediatr Infect Dis J*, 2025, **44**(3): 200-206
- [6] Sun B, Ling Y, Li J, *et al.* Advances in adhesion-related pathogenesis in *Mycoplasma pneumoniae* infection. *Front Microbiol*, 2025, **16**: 1613760
- [7] Viz-Lasheras S, Gómez-Carballa A, Bello X, *et al.* A diagnostic host-specific transcriptome response for *Mycoplasma pneumoniae* pneumonia to guide pediatric patient treatment. *Nat Commun*, 2025, **16**(1): 673
- [8] Becker A, Kannan T R, Taylor A B, *et al.* Structure of CARDS toxin, a unique ADP-ribosylating and vacuolating cytotoxin from *Mycoplasma pneumoniae*. *Proc Natl Acad Sci USA*, 2015, **112**(16): 5165-5170
- [9] Ramasamy K, Balasubramanian S, Kirkpatrick A, *et al.* *Mycoplasma pneumoniae* CARDS toxin exploits host cell endosomal acidic pH and vacuolar ATPase proton pump to execute its biological activities. *Sci Rep*, 2021, **11**(1): 11571
- [10] Ahn J G, Cho H K, Li D, *et al.* Efficacy of tetracyclines and fluoroquinolones for the treatment of macrolide-refractory *Mycoplasma pneumoniae* pneumonia in children: a systematic review and meta-analysis. *BMC Infect Dis*, 2021, **21**(1): 1003
- [11] Wei J, Walker A S, Eyre D W. Addition of macrolide antibiotics for hospital treatment of community-acquired pneumonia. *J Infect Dis*, 2025, **231**(4): e713-e722
- [12] Lee H, Yun K W, Lee H J, *et al.* Antimicrobial therapy of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. *Expert Rev Anti Infect Ther*, 2018, **16**(1): 23-34
- [13] Chen Y C, Hsu W Y, Chang T H. Macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric community-acquired pneumonia. *Emerg Infect Dis*, 2020, **26**(7): 1382-1391
- [14] Oishi T, Ouchi K. Recent trends in the epidemiology, diagnosis, and treatment of macrolide-resistant *Mycoplasma pneumoniae*. *J Clin Med*, 2022, **11**(7): 1782
- [15] Dai F F, Liu F Q, Chen X, *et al.* The treatment of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. *J Clin Pharm Ther*, 2021, **46**(3): 705-710
- [16] Yang H J, Song D J, Shim J Y. Mechanism of resistance acquisition and treatment of macrolide-resistant *Mycoplasma pneumoniae* pneumonia in children. *Korean J Pediatr*, 2017, **60**(6): 167-174
- [17] Li L, Ma J, Guo P, *et al.* Molecular beacon based real-time PCR p1 gene genotyping, macrolide resistance mutation detection and clinical characteristics analysis of *Mycoplasma pneumoniae* infections in children. *BMC Infect Dis*, 2022, **22**(1): 724
- [18] Zhang G, Li Q, Zhang J. Clinical effects of traditional Chinese medicine + azithromycin in the treatment of mycoplasma pneumonia. *Int J Gen Med*, 2025, **18**: 825-833
- [19] Sun R, Zhang Y, Cheng F, *et al.* Effect of pulmonary rehabilitation nursing approach with integrated traditional Chinese and western medicine in children with mycoplasma pneumonia after bronchoalveolar lavage. *Pak J Med Sci*, 2025, **41**(4): 1105-1109
- [20] Sun J H, Sun F, Yan B, *et al.* Data mining and systematic pharmacology to reveal the mechanisms of traditional Chinese medicine in *Mycoplasma pneumoniae* pneumonia treatment. *Biomedicine Pharmacother*, 2020, **125**: 109900
- [21] Peng K, Liao Y, Li X, *et al.* Vimentin is an attachment receptor for *Mycoplasma pneumoniae* P1 protein. *Microbiol Spectr*, 2023, **11**(2): e0448922
- [22] Jiang Z, Li S, Zhu C, *et al.* *Mycoplasma pneumoniae* infections: pathogenesis and vaccine development. *Pathogens*, 2021, **10**(2): 119
- [23] Bliersch K F, Burchert J P, August S C, *et al.* Structural model of the M7G46 methyltransferase TrmB in complex with tRNA. *RNA Biol*, 2021, **18**(12): 2466-2479
- [24] Kim M, Park S, Lee S J. Global transcriptional regulator TrmB family members in prokaryotes. *J Microbiol*, 2016, **54**(10): 639-645
- [25] Medina J L, Coalson J J, Brooks E G, *et al.* *Mycoplasma pneumoniae* CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation. *Am J Respir Cell Mol Biol*, 2012, **46**(6): 815-822
- [26] Kannan T R, Coalson J J, Cagle M, *et al.* Synthesis and distribution of CARDS toxin during *Mycoplasma pneumoniae* infection in a murine model. *J Infect Dis*, 2011, **204**(10): 1596-1604
- [27] Chaudhry R, Ghosh A, Chandolia A. Pathogenesis of *Mycoplasma pneumoniae*: an update. *Indian J Med Microbiol*, 2016, **34**(1): 7-16
- [28] Fu B B, Zhong L L, Ye T T, *et al.* Value of autotaxin in predicting refractory *Mycoplasma pneumoniae* pneumonia in children and its correlation with inflammatory cytokines. *Chin J Contemp Pediatr*, 2022, **24**(7): 765-770
- [29] Fang W, Huang J, Wang J, *et al.* Blockade of interleukin-6 receptor attenuates apoptosis and modulates the inflammatory response in

- Mycoplasma pneumoniae* infected A549 cells. Am J Transl Res, 2022, **14**(9): 6187-6195
- [30] Chen M, Deng H, Zhao Y, *et al.* Toll-like receptor 2 modulates pulmonary inflammation and TNF- α release mediated by *Mycoplasma pneumoniae*. Front Cell Infect Microbiol, 2022, **12**: 824027
- [31] Tian F, Chen L P, Yuan G, *et al.* Differences of TNF- α , IL-6 and Gal-3 in lobar pneumonia and bronchial pneumonia caused by *Mycoplasma pneumoniae*. Technol Health Care, 2020, **28**(6): 711-719
- [32] Ding Y, Chu C, Li Y, *et al.* High expression of HMGB1 in children with refractory *Mycoplasma pneumoniae* pneumonia. BMC Infect Dis, 2018, **18**(1): 439
- [33] Ledford J G, Lo B, Kislán M M, *et al.* Surfactant protein-a inhibits mycoplasma-induced dendritic cell maturation through regulation of HMGB-1 cytokine activity. J Immunol, 2010, **185**(7): 3884-3894
- [34] Kumar S. *Mycoplasma pneumoniae*: a significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. Indian J Med Res, 2018, **147**(1): 23-31
- [35] Ding G, Zhang X, Vinturache A, *et al.* Challenges in the treatment of pediatric *Mycoplasma pneumoniae* pneumonia. Eur J Pediatr, 2024, **183**(7): 3001-3011
- [36] Jensen P S, Halber M D, Putman C E. Mycoplasma pneumonia. CRC Crit Rev Diagn Imaging, 1980, **12**(4): 385-415
- [37] Aziz N, Kim M Y, Cho J Y. Anti-inflammatory effects of luteolin: a review of *in vitro*, *in vivo*, and *in silico* studies. J Ethnopharmacol, 2018, **225**: 342-358
- [38] Jiang Z B, Wang W J, Xu C, *et al.* Luteolin and its derivative apigenin suppress the inducible PD-L1 expression to improve anti-tumor immunity in KRAS-mutant lung cancer. Cancer Lett, 2021, **515**: 36-48

木犀草素抗肺炎支原体的作用及机制研究*

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摘要 目的 本研究旨在探讨木犀草素对肺炎支原体(MP)的抑制作用, 并阐明其潜在作用机制。**方法** 采用网络药理学方法从金荞麦多酚提取物中筛选出木犀草素为主要活性成分, 考察其对2株肺炎支原体菌株的作用效果。采用具有可视化特征的改良培养基测定木犀草素的最低抑菌浓度(MIC)。通过qRT-PCR检测木犀草素作用后, MP生长及致病性相关关键蛋白的表达水平。同时比较木犀草素处理组与未处理组对MP感染A549细胞活力的影响。利用小鼠模型评价体内抗MP活性, 并分析肺组织中炎症细胞因子的表达。**结果** 木犀草素可有效抑制2株MP菌株, 对M19与M129的MIC₉₀值均为100 mg/L。木犀草素处理可显著下调2株菌株中黏附蛋白P1、P30的表达。而P65、HMW3、TrmB及CARDS TX的表达仅在M19菌株中经木犀草素干预后出现下调。木犀草素还可提高MP感染A549细胞的生长与活力。小鼠模型中, 木犀草素给药后小鼠体重平稳增长, 耐受性良好。木犀草素在肺组织中的抑菌率达50.7%, 显著高于罗红霉素组的25.2%。此外, 木犀草素可降低MP感染小鼠体内IL-6、TNF- α 、HMGB1等炎症因子的表达。**结论** 木犀草素通过下调毒性相关蛋白(P1、P30、P65、HMW3、TrmB、CARDS TX)的表达、调控宿主炎症反应, 安全有效地抑制MP尤其是耐药株M19的增殖与致病性。研究结果提示, 木犀草素有望为MP感染, 特别是耐药菌株所致感染, 提供新的治疗策略。

关键词 肺炎支原体, 木犀草素, 黏附蛋白, CARDS TX, TrmB

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