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Chemical constituents and their anti-infective activity of *Paeonia suffruticosa*

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Author contribution

Yue-Mao shen was responsible for design and concept of paper, Chun-Hua Lu was responsible for writing of manuscript, Yang Wu was responsible for making figure and structures of chemical constituents, Si-Yun Liu was responsible for the provision of herbs.

Competing interests

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

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Abbreviations

P. Suffruticosa, *Paeonia suffruticosa*; T3SS, type III secretion system; *S. typhimurium*, *Salmonella enterica* serovar typhimurium UK-1 γ 8956; LB, Luria-Bertani; SPI-1, Salmonella pathogenicity island 1; TLC, Thin-layer chromatography; DMSO, Dimethyl sulfoxide; MIC, Minimum inhibitory concentration.

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Abstract

Objective: Research the chemical constituents of *Paeonia suffruticosa* that are responsible for its anti-infective properties. **Methods:** Several column chromatographic methods were used to purify the chemical constituents from *P. suffruticos*a, including medium pressure liquid chromatography, Sephadex LH-20, and normal silica gel. A disc diffusion method was used to screen for antibacterial activity, and their anti-virulence activity was assessed on the type III secretion system (T3SS) of Salmonella pathogenicity island 1 (SPI-1) in *Salmonella enterica* serovar Typhimurium UK-1 γ 8956 by SDS-PAGE and western blots. **Results**: Twenty-one compounds were identified. Compounds **7, 8** and **17** showed moderate activity against *S. aureus* ATCC25923, compounds **8**, **9** and **10** showed weak activities against *B. subtilis* ACCC11060. Meanwhile, phenols (**14–18**) and flavonoids (**20** and **21**) inhibited **T3SS** protein secretion of *S. typhimurium* without affecting bacterial growth. Furthermore, a strong inhibitory effect was observed for **17** and **20** on SPI-1 mediated invasion of HeLa cells. Additionally, no toxicity was observed for these compounds. **Conclusion**: *P. suffruticosa* has anti-infective properties due in part to the fact that phenols and flavonoids can block the secretion of T3SS-associated protein effectors.

Keywords: *Paeonia suffruticosa*; anti-infective activity; type III secretion system; phenols and flavonoids

Highlight

Twenty-one compounds including a new one were isolated from the root extract of *Paeonia suffruticosa*. Those compounds showed weak antibacterial activities. Whereas, phenols and flavonoids especially the main constituent paeonol showed anti-virulence activity against T3SS of Salmonella. Therefore, this study provide the reasonable evidence for the medicinal use of *P. suffruticosa*.

Background

Paeonia suffruticosa Andrews (*P. suffruticosa*) also known as the "moutan" or "tree peony", is one of the most commonly used medicinal plants in China [1, 2]. The roots of *P. suffruticosa* have been used in traditional medicine to treat tumors, inflammation, and hypertension [3, 4]. They also have been used as a remedy for important Chinese medicine such as Liu Wei Di Huang Pill. More than 260 compounds including monoterpenoids and their glucosides, triterpenoids, flavonoids, phenols, paeonol, and resveratrol have been identified in the genus of Paeonia [5–8]. Pharmacological studies have shown that "Moudan Cortex" has the function of anti-inflammatory, antibacterial, anti-infection, etc.[9–14]. Those molecules and extracts attenuate infection but do not exhibit significant bactericidal activity, such as Nadin Marwan Almosnid et al. reported that the monoterpene glycosides, 6-O-vanillyoxypaeoniflorin, mudanpioside H and galloyl-oxypaeoniflorin, showed moderate antibacterial activity with MIC values of 100 to 500 μg/mL; Zhou et al. reported the buds extract showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* O157:H7 with MIC of 1.56 mg/mL and 6.25 mg/mL [10, 14]. Therefore, the antibacterial and anti-infection active constituents and mechanisms still need to be screened and characterized, especially effective against clinical pathogens [15].

Developing anti-virulence agents against multi-drug resistant bacteria is an attractive strategy because of its non-bacterial feature [16]. Among them, type III secretion system (T3SS), the protein transport needle-like nano-machines, can mediate interactions between Gram-negative bacteria and their hosts, and therefore, play an essential role in the infection process.

In this experiment, we identified 21 compounds (**1–21**) from the ethanol extract of *P. suffruticosa*. Further, we screened their antimicrobial activity using the disc diffusion method and their anti-virulence activity against T3SS by SDS-PAGE and western blot in vitro. Our results suggested that phenols and flavonoids can block the secretion of T3SS-related protein effectors such as SipA, SipB, SipC, and SipD. However, none exhibited excellent antibacterial activity, which explains the anti-infective properties of *P. suffruticosa*.

Materials and methods

Plant materials

The *P. suffruticosa* roots were purchased from Anhui Tongling, Peony trade net Co Ltd (China).

Culture of the strains

The bacterial strains including *Salmonella enterica* serovar Typhimurium UK-1 γ 8956 (*S. typhimurium*), *Proteusbacillus vulgaris* CPCC160013, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ACCC11060 were used in this study. All the bacterial strains were grown on Luria-Bertani (LB) agar media (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.4). *S. typhimurium* was cultured in LB broth or on LB agar plates supplemented with 0.2% L-arabinose at 37°C/220 rpm or 25°C/ 220 rpm [17].

Compounds isolation

The air-dried *P. suffruticosa* roots (1.0 kg) were smashed and then extracted with ethyl acetate (EA) at room temperature 3 times. A rotary evaporator evaporated the extract. Depending on these compounds' polarity and molecular weight, we used medium pressure liquid chromatography, high performance liquid chromatography and Sephadex LH-20 interleaved. The EA extract was subjected to medium pressure liquid chromatography over RP-18 silica gel eluted with 30, 50, 70, 90 and 100% MeOH (v/v) and 20 fractions were obtained, respectively. According to the thin-layer chromatography (TLC) results, 4 fractions (Fr.1–4) were obtained. Compounds **1** (5.0 g), **3** (4.8 mg), **6** (20 mg), **13** (8.3 mg), **14** (4.8 mg) and **16** (24.8 mg) were purified by column chromatography (RP-18, Sephadex LH-20, and silica gel) from Fr.1. Fr.2 was subjected to column chromatography over Sephadex LH-20 eluted with MeOH and then were purified by high performance liquid chromatography (Waters 2545) to obtain **8** (12 mg), **9** (4.5 mg) and **15** (16.4 mg) were purified. In the same way, **5** (44.2 mg), **7** (78 mg), **18** (9.3 mg), **20** (6.2 mg) and **21** (5.2 mg) were separated from the Fr.3 and compound **17** (2.8 g) was purified from Fr.4.

Another 1 kg of air-dried *P. suffruticosa* roots were extracted with 70% EtOH. By using the method of liquid-liquid extraction, the BuOH extract was obtained and further purified to obtain **2** (10.1 mg), **4** (8.8 mg), **10** (6.5 mg), **11** (9.2 mg), **12** (35.8 mg) and **19** (7.5 mg).

Disc diffusion method

The antibacterial activities of compounds (**1–21**) against *S. typhimurium*, *S. aureus* ATCC25923, *P. vulgaris* CPCC160013 and *B. subtilis* ACCC11060 were detected via a paper disc diffusion assay [18]. Compounds under test were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL. These compounds were uptaken at a concentration of 80 μg/disc onto 6 mm diameter paper discs and laid on the surface of the agar medium. Gentamycin was used as a positive control. The assay plates were cultured at 37°C for 24h and checked for the presence of an inhibition zone.

Profiling of secreted protein in bacterial culture supernatants

The effects of compounds **1–21** on the secretion of Salmonella pathogenicity island 1 (SPI-1) effector proteins at a concentration of 100 μM were examined. Csn-B was used as the positive control. Negrea et al. and Li et al. with some modifications were used to isolate and detect the secreted protein in bacterial culture supernatants [19, 20]. Briefly, 1:10 dilution of *S. typhimurium* overnight cultures in LB (0.2% L-arabinose) for 4 h with or without compounds at indicated concentrations at 37°C /220 rpm. Secreted proteins in the 1 mL culture supernatant were precipitated with a final concentration of 10% cold trichloroacetic acid (TCA) and then centrifuged at 12,000 *g* for 10 min at 4°C and washed with 500 μL cold acetone. The precipitation was repeated twice and dried for 15 min. The proteins were denatured and separated via 10% SDS-PAGE and stained with Coomassie blue.

Western blot analysis

Western blot was performed as described previously [20].

Bacterial growth curve

S. typhimurium was amplified in LB at 37°C /220 rpm for 12h, then diluted 1:100 in fresh LB and incubated with the compounds at the indicated concentrations for 7 h. The OD_{600} of the cultures was measured at the indicated time points using a microbial plate reader (Bio-Rad 680, USA). Three replicate samples were used for each experiment.

Cytotoxicity assay

The sulforhodamine B (SRB) method was used to determine the effect of compounds on the growth rate of host cells [21]. HeLa cells were seeded in 96-well plates at a density of 4×10^4 cells per well and incubated overnight at 37°C in a 5% CO_2 incubator. Then, cells were treated with different concentrations of compounds **17**, **18**, **20** and also set the positive control and blank control with isovolumetric DMSO and Dulbecco's modified eagle medium (DMEM) (10% Fetal bovine serum- FBS) for 12 h at 37°C. Discard the culture medium

softly, cells were fixed with the presence of 100 μL 10% TCA in advance of cold. Allow standing for more than 1 h. After removing the solution of TCA, distilled water slowly washes 5 times. Cells were stained with 100 μL staining solution of SRB and incubated 30 min at room temperature. Discard the SRB, 1% acetic acid washes 5 times to remove the unbound staining solution. 100 μL 10 mM tris (pH 10.0) was added to the 96-well plates for dissolving the remaining SRB. The Microplate Reader tested the OD_{570} value.

Detection of SipC in the invasion assay

HeLa cells were seeded at the density of 2×10^6 in 50 mm cell culture plates and incubated at 37°C, and 5% CO_2 in DMEM culture media supplemented with 10% FBS for 16 h. *S. typhimurium* was incubated with the compound of 100 μM at 37°C for 4 h. HeLa cells were FBS starved for 30 min and then infected with Salmonella at a multiplicity of infection of 50 for 10, 25, and 40 min at 37°C also with the addition of those compounds. The cell pellet was collected and lyzed in 200 μL radio Immunoprecipitation assay (RIPA) lysis buffer. The cell lysate was centrifuged at 13, 000 *g*, 5 min at 4°C. The supernatant was denatured and detected by western blot. All experiments were performed in triplicates.

Results

Structure determination of compounds 1–21

Twenty-one compounds (**1–21,** Table S1) were purified from the roots extract of *P. suffruticosa* (1.0 kg) by repeated column chromatography of medium pressure liquid chromatography, Sephadex LH-20, normal silica gel, and high performance liquid chromatography. Their structures were determined based on their 1D, 2D nuclear magnetic resonance (NMR), and electrospray ionization mass spectrometry (ESIMS) data and compared with those reported. They belonged to monoterpenes or their glycosides (**1–8, 10–12**), alkaloids (**9**), phenols (**13–19**) and flavonoids (**20–21**). Their structures, names, formulas, and related references are listed in Table S1 and their ^1H and ^{13}C NMR spectra are provided in Figure S1–S27. Speak with the emphasis here are compounds **11** and **12**, they were first isolated as natural products from the genus Paeonia, and **12** is a new one. The 1D, 2DNMR spectra and ESIMS of **12** were provided in Table S2 (Figure S1–S7).

Antibacterial activity

The disc diffusion assay indicated that compounds **7**, **8**, and **17** showed moderate activity against *S. aureus* ATCC 25923 at 80 μg/disc with an inhibition zone of 10, 8, and 10 mm, respectively. Compounds **8**, **9**, and **10** showed weak activities against *B. subtilis* ACCC11060 at 80 μg/disc with the inhibition zone of 12, 10, and 8 mm. Paeonol (**17**) was the main constituent (2.8 g from 1 kg root extract) of Paeonia. It was considered to be the active antibacterial constituent, but our results suggested that paeonol showed no antibacterial activities against tested strains at 80 μg/disc [11].

Compounds 14–18 and 20–21 inhibited the secretion of SPI-1 effector proteins and 17 and 20 also blocked the invasion of *S. typhimurium* into HeLa cells

When grown in temperature-induced conditions, *S. typhimurium* can secrete SPI-1 effector proteins into the culture supernatant, which are used to assist them in invading and infecting eukaryotic cells [20]. Among the 21 compounds, **14–18** and **20–21** exhibited specific inhibitory effects on the secretion of the T3SS effectors SipA, SipB, SipC, and SipD without affecting FliC (Figure 1A). Furthermore, we tested the effects of **14–18** and **20–21** on the growth of Salmonella. The results (Figure 1C) suggested that none of the seven compounds affected the growth of *S. typhimurium* at a concentration of 100 μM, which indicated that they might be virulence inhibitors.

Therefore, compounds **17** and **20** (Figure 1B) were further evaluated for the effects on SPI-1 mediated invasion. Firstly, the SRB method measured the cytotoxicity of **17** and **20** on HeLa cells. The results suggested that no apparent toxic effects were observed compared to the DMSO control at several concentrations of less than 100 μM during a 4 h incubation (Figure 2A). Secondly, the inhibitory effects of **17** and **20** on the invasion of HeLa cells were assessed. After Salmonella infection of HeLa cells, western blot showed that the SipC levels were barely visible in the medium and abundant in HeLa cells, indicating that Salmonella was able to successfully invade HeLa cells. The presence of **17** and **20** significantly reduced the content of SipC in HeLa cells compared to the DMSO control (Figure 2B). Therefore, **17** and **20** can reduce the invasive ability of Salmonella on HeLa cells.

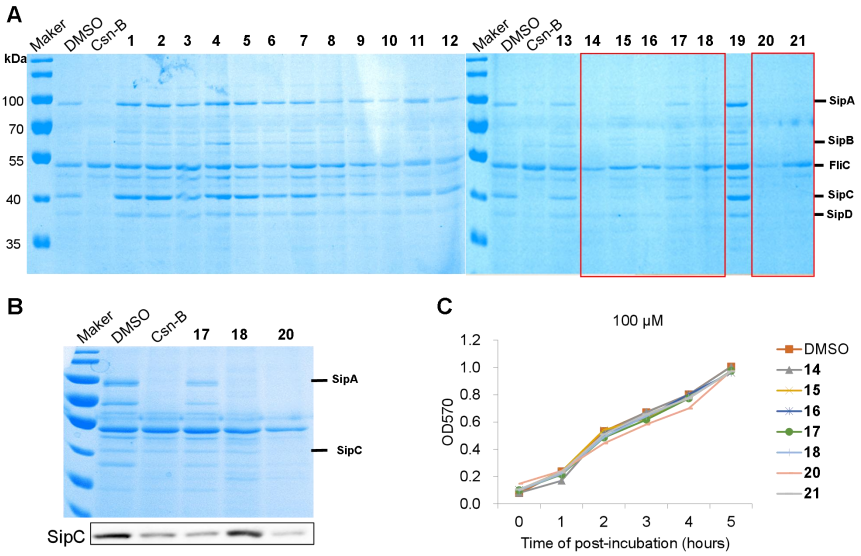

Figure 1 Compounds inhibited the secretion of SPI-1 effectors and did not affect the growth of *S. typhimurium*. (A) The inhibitory activities of compounds 1–21 (100 μM, respectively) against the secretion of SPI-1 effector proteins of *S. typhimurium*. SipA/B/C/D, SPI-1 effector proteins; FliC, flagellar filament protein; M, marker; C, Csn-B positive control; D, DMSO control; (B) The Coomassie blue staining or Western blotting results of compounds **17**, **18** and **20** inhibited secretion of SPI-1 effector proteins; (C) Compounds at a concentration of 100 μM did not significantly inhibit the bacterial growth. SPI-1, Salmonella Pathogenicity Island 1.

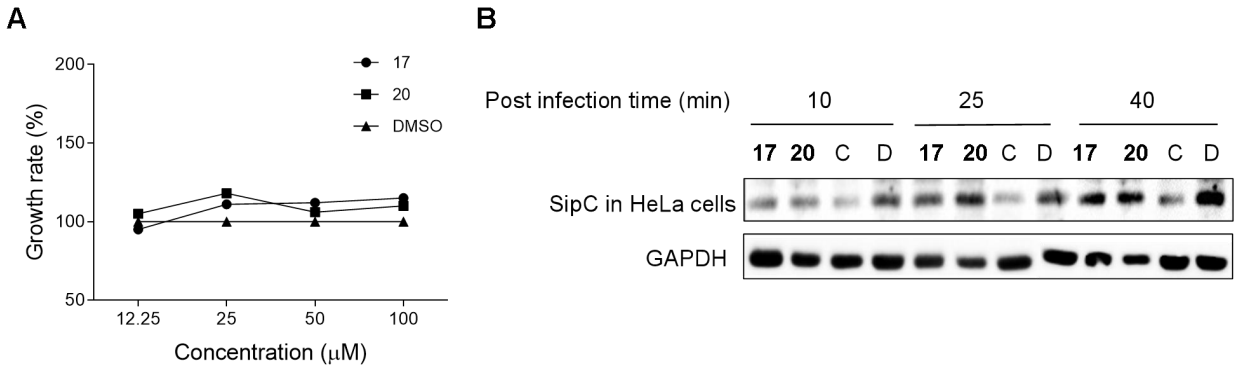

Figure 2 Compounds 17 and 20 inhibit the invasion of *S. typhimurium* into HeLa cells. (A) Compounds **17** and **20** at a range of concentrations from 12.25 to 100 μM did not significantly inhibit the growth of HeLa cells; (B) The western blot results of inhibitory effects of **17** and **20** on the invasion of *S. typhimurium* into HeLa cells at a final concentration of 100 μM with MOI 50.

Discussion

Although some antimicrobial properties of monoterpene glycosides, paeonol, benzoic acid, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-beta-d-glucopyranose from Paeonia and the crude extract have been reported, the MIC reported by different studies varied ranging from 100 to 500 μg/mL [11, 14, 15, 22].

Our antibacterial assay of compounds **1–21** also suggested that monoterpenoids and their glycosides (**1–8, 10–12**), alkaloid (**9**), phenols (**13–19**) and flavonoids (**20** and **21**) showed no evident activities against all tested bacterial strains at the concentration of 80 μg/disc. The results suggested that all the isolated compounds are not good bactericides.

By studying the secretion of T3SS proteins associated with bacterial virulence, we have found phenols (**14–18**) and flavonoids (**20–21**), especially **17** and **20**, can inhibit the secretion of effector proteins rather than the growth of *S. typhimurium*, and also inhibit SPI-1 effect SipC into HeLa cells, which provided an explanation for the anti-infective properties of *P. suffruticosa*.

The inhibitory effect of plant phenols and flavonoids on the secretion of the SPI-1 effector proteins have been reported [23–25]. Our results further suggest that the phenols and flavonoids from *P. suffruticosa*, such as **17** and **20** also block the SPI-1 T3SS virulence effector, which may be responsible for the anti-infective activity of *P. suffruticosa*.

Conclusions

In conclusion, 21 compounds (Table S1) were obtained from the root extract of *P. suffruticosa*. The antibacterial bioassay demonstrated that **1–21** were not good bactericides. The anti-infective property of *P. suffruticosa* should be due to inhibiting the secretion of the T3SS effectors of some related compounds such as **17** and **20**. Compound **17** (paeonol) is one of the main active ingredients in the root bark of *P. suffruticosa* and an important index for the quality control of these materials. Compound **20** (quercetin) and flavonoids with 1, 2-catechol motifs have been reported as SPI-1 T3SS inhibitors. Although a number of medicinal plants have been used to treat infections by enteric bacterial pathogens, the action mechanisms have been unclear. Focusing on T3SS responsible for bacterial virulence, we have found phenols and flavonoids can explain the traditional usage of *P. suffruticosa* as anti-infective properties.

References

1. Hong MH, Kim JH, Na SH, et al. Inhibitory effects of Paeonia suffruticosa on allergic reactions by inhibiting the NF-kappaB/I kappaB-alpha signaling pathway and phosphorylation of ERK in an animal model and human mast cells. *Biosci Biotechnol Biochem* 2010;74(6):1152–1156. Available at: http://dx.doi.org/10.1271/bbb.90676
2. He CN, Peng Y, Zhang YC, et al. Phytochemical and biological studies of paeoniaceae. *Chem Biodivers* 2010;7(4):805–838. Available at: http://dx.doi.org/10.1002/cbdv.200800341
3. Ha do T, Trung TN, Hien TT, et al. Selected compounds derived from Moutan Cortex stimulated glucose uptake and glycogen synthesis via AMPK activation in human HepG2 cells. *J Ethnopharmacol* 2010;131(2):417–424. Available at: http://dx.doi.org/10.1016/j.jep.2010.07.010
4. Dai J, Liang K, Zhao S, et al. Chemoproteomics reveals baicalin activates hepatic CPT1 to ameliorate diet-induced obesity and hepatic steatosis. *Proc Natl Acad Sci U S A* 2018;115(26):E5896–E5905. Available at: http://dx.doi.org/10.1073/pnas.1801745115
5. Zhao DD, Jiang LL, Li HY, Yan PF, Zhang YL. Chemical components and pharmacological activities of terpene natural products from the Genus Paeonia. *Molecules* 2016;21(10):1362–1376. Available at: http://dx.doi.org/10.3390/molecules21101362
6. Almosnid NM, Gao Y, He C, Park HS, Altman E. In vitro antitumor effects of two novel oligostilbenes, cis- and trans-suffruticosol D, isolated from Paeonia suffruticosa seeds. *Int J Oncol* 2016;48(2):646–656. Available at: http://dx.doi.org/10.3892/ijo.2015.3269
7. Gao Y, He C, Ran R, et al. The resveratrol oligomers, cis- and trans-gnetin H, from Paeonia suffruticosa seeds inhibit the growth of several human cancer cell lines. *J Ethnopharmacol* 2015;169:24–33. Available at: http://dx.doi.org/10.1016/j.jep.2015.03.074
8. He CN, Peng Y, Wu QL, et al. Simultaneous determination of ten stilbenes in the seeds of Paeonia species using Hplc-Dad. *J Liq Chromatogr R T* 2013;36(12):1708–1724. Available at: http://dx.doi.org/10.1080/10826076.2012.698674
9. Wu KC, Lee DY, Hsu JT, et al. Evaluations and mechanistic interrogation of natural products isolated from *Paeonia suffruticosa* for the treatment of inflammatory bowel disease. *Front Pharmacol.* 2021;12:696158. Available at: http://dx.doi.org/10.3389/fphar.2021.696158
10. Zhou Y, Zhang Y, Zong H, et al. Chemical constituents, antibacterial activity and mechanism of *Paeonia suffruticosa* Andr. buds extract against *Staphylococcus aureus* and *Escherichia coli* O157:H7. *Nat Prod Res* 2021;35(6):1005–1009. Available at: http://dx.doi.org/10.1080/14786419.2019.1610961
11. Papandreou V, Magiatis P, Chinou I, et al. Volatiles with antimicrobial activity from the roots of Greek Paeonia taxa. *J Ethnopharmacol* 2002;81(1):101–104. Available at: http://dx.doi.org/10.1016/S0378-8741(02)00056-9

12. Jung J, Bae KH, Jeong CS. Anti-Helicobacter pylori and antiulcerogenic activities of the root cortex of *Paeonia suffruticosa*. *Biol Pharm Bull* 2013;36(10):1535–1539. Available at: http://dx.doi.org/10.1248/bpb.b13-00225
13. Tian YZ, Wang Y, Liu JZ, et al. Determination of two main components in bark of *Paeonia suffruticosa* by HPLC. Zhongguo Zhong Yao Za Zhi. 2005;30(16):1265–1268. Available at: https://search.bvsalud.org/gim/resource/pt/wpr-239705
14. An RB, Kim HC, Lee SH, et al. A new monoterpene glycoside and antibacterial monoterpene glycosides from *Paeonia suffruticosa*. *Arch Pharm Res* 2006;29(10):815–820. Available at: http://dx.doi.org/10.1007/BF02973899
15. Ngan LT, Moon JK, Shibamoto T, Ahn YJ. Growth-inhibiting, bactericidal, and urease inhibitory effects of *Paeonia lactiflora* root constituents and related compounds on antibiotic-susceptible and -resistant strains of *Helicobacter pylori*. *J Agric Food Chem* 2012;60(36):9062–9073. Available at: http://dx.doi.org/10.1021/jf3035034
16. Gauthier A, Finlay BB. Type III secretion system inhibitors are potential antimicrobials - Present in disease-causing gram-negative bacteria, components of this system might be good targets for novel antimicrobial agents. *Asm News* 2002;68(8):383–387.
17. Curtiss R 3rd, Wanda SY, Gunn BM, et al. *Salmonella enterica* serovar typhimurium strains with regulated delayed attenuation in vivo. *Infect Immun* 2009;77(3):1071–1082. Available at: http://dx.doi.org/10.1128/IAI.00693-08
18. Raahave D. Paper disk-agar diffusion assay of penicillin in the presence of streptomycin. *Antimicrob Agents Chemother* 1974;6(5):603–605. Available at: http://dx.doi.org/10.1128/AAC.6.5.603
19. Negrea A, Bjur E, Ygberg SE, et al. Salicylidene acylhydrazides that affect type III protein secretion in *Salmonella enterica* serovar typhimurium. *Antimicrob Agents Chemother* 2007;51(8):2867–2876. Available at: http://dx.doi.org/10.1128/AAC.00223-07
20. Li J, Lv C, Sun W, et al. Cytosporone B, an inhibitor of the type III secretion system of *Salmonella enterica* serovar Typhimurium. *Antimicrob Agents Chemother* 2013;57(5):2191–2198. Available at: http://dx.doi.org/10.1128/AAC.02421-12
21. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990;82(13):1107–1112. Available at: http://dx.doi.org/10.1093/jnci/82.13.1107
22. Ding F, Qiu S, Guo L, Gong Z, Yang X. Research on the in vitro growth inhibition effect of *Paeonia suffticosa* Andr, (PSA) on bacteria. Lishizhen Medicine and Materia Medica Research. 2008;14(8):452. Available at: 10.3969/j.issn.1008-0805.2003.08.003
23. Li Y, Hutchins W, Wu X, et al. Derivative of plant phenolic compound inhibits the type III secretion system of Dickeya dadantii via HrpX/HrpY two-component signal transduction and Rsm systems. *Mol Plant Pathol* 2015;16(2):150–163. Available at: http://dx.doi.org/10.1111/mpp.12168
24. Fan SS, Tian F, Li JY, et al. Identification of phenolic compounds that suppress the virulence of *Xanthomonas oryzae* on rice via the type III secretion system. *Mol Plant Pathol* 2017;18(4):555–568. Available at: http://dx.doi.org/10.1111/mpp.12415
25. Tsou LK, Lara-Tejero M, RoseFigura J, et al. Antibacterial flavonoids from medicinal plants covalently inactivate type III protein secretion substrates. *J Am Chem Soc* 2016;138(7):2209–2218. Available at: http://dx.doi.org/10.1021/jacs.5b11575