Anti-bacterial effect of phytoconstituents isolated from *Alimatis rhizoma*

Chengfu Li1, Wei Yan1,2, Enji Cui1* and Changji Zheng1*

**Abstract**

Five compounds including three triterpenoids and two sesquiterpenes were isolated from *Alimatis rhizoma*. Their chemical structures were determined to be alisol B 23-acetate (1), alisol C 23-acetate (2), alisol B (3), alismol (4) and alismoxide (5) by various spectroscopic analysis, including 1H-NMR, 13C-NMR, HMBC and MS spectra. Compounds 1–5 were evaluated for their antibacterial potential against 6 strains of bacteria including three drug-resistant bacteria (one methicillin-resistant *Staphylococcus aureus* strain CCARM 3506, two quinolone-resistant *Staphylococcus aureus* strains CCARM 3505 and CCARM 3519), two G+ bacteria (*Streptococcus mutans* KCTC 3289 and *Staphylococcus aureus* KCTC 209) and one G− bacterium (*Escherichia coli* KCTC 1924). Compounds 1–5 showed strong antibacterial effect against *S. mutans* KCTC 3289, their MIC values were 2, 64, 16, 32 and 32 μg/mL, respectively. The antibacterial activity results of compounds 1–5 against these bacteria were reported for the first time. The results indicate that *Alimatis rhizoma* are potential sources of new antibacterial material.

**Keywords** *Alimatis rhizoma*, Phytoconstituents, Antibacterial activity, Structure–active relationship

**Introduction**

Since the advent of antibiotics, it has cured countless infected patients around the world, but with the passage of time and people’s abuse of antibiotics, it has led to the emergence of drug-resistant strains. The original strong antibacterial effect is getting worse, even some antibiotics have been loss of function which seriously threatens human life [1]. Today the drug resistance crisis is becoming more and more serious, and has became one of the biggest threats facing the global society. If the trend of bacterial resistance is not effectively controlled, human will fall into the crisis of no drugs available, so it is urgent to separate active compounds with good antibacterial activity from natural medicines.

*Alimatis rhizoma*, the dried tuber of *Alisma orientalis*, is a widely used traditional herbal medicine in China, and broadly distributed in Fujian, Sichuan, Jiangxi, Yunnan province and other areas of China. Triterpenes and sesquiterpenes [2] were typical metabolites of *A. rhizoma*, also it contained diterpenes, volatile oils and alkaloids. *A. rhizoma* had various pharmacological effects including lowering blood lipids [3], anti-urolithiatic effect [4], anti-inflammatory effect [5, 6], anti-fatty liver effect [7, 8], diuretic effect [9], protecting cardiovascular system [10] and anti-cancer effects [11, 12].

In this research, we demonstrated the isolation and structural characterization of three protostane-type triterpenoids (1–3) and two guaiane-type sesquiterpenes (4, 5) from the methanolic extract of *A. rhizoma*. All compounds were explored for antibacterial activity test.

**Materials and methods**

**Plant materials**

*Alimatis rhizoma* were purchased from YanBian Wei Ye pharmacy in YanJi and Prof. Hui-Zi Lv of College of Pharmacy, YanBian University, YanJi, China identified.
A voucher specimen (YBU-R012) was stored at the Medicinal Chemistry of Natural Products Laboratory, YanBian University, YanJi, China.

**Instruments and reagents**

NMR spectra were recorded on a Bruker 300 MHz (AV 300, Germany). Mass spectra were measured on a time of flight-mass spectrometry (TOF-MS, AXIMA-QIT, Shimadzu, Tokyo, Japan). Column chromatography was performed using silica gel (200–300 mesh, Qingdao Haiyang, Qingdao, China) and octadecylsilyl (ODS) silica gel (Kieselgel 60, Art 7734, Merck, Germany). Thin-layer chromatography (TLC) on pre-coated silica gel GF254 (200 × 200 mm, Branch of Qingdao Haiyang Chemical, Qingdao, China) and ODS (Kieselgel 60 F254, Art 5714, Merck, Germany) were carried out [13].

**Extraction and isolation of A. rhizoma**

The air-dried powdered *A. rhizoma* (5 kg) was soaked in methanol (MeOH), then obtained 298 g of methanol extract by filtration and concentration. The extract was dissolved with 0.5 L of distilled water, and successively fractionated with petroleum ether (PE, 0.5 L × 3) and ethyl acetate (EtOAc, 0.5 L × 3). The concentrated EtOAc extract (50.89 g) was applied to normal phase silica gel (SiO2) column chromatography (CC), then fully eluted with a gradient of dichloromethane (CH2Cl2)-MeOH (1:0–0:1) to obtain 13 fractions (EA–EM). Fr.EF (11.22 g) was subjected to SiO2 column, and gradient elution was performed using PE-EtOAc (100:1–0:1) to get 5 fractions (Fr.EF1–EF5). Compound 1 (100 mg) was obtained from Fr.EF3. Fr.EF4 (5.54 g) was further subjected to SiO2 CC with CH2Cl2–MeOH (20:1–0:1) to yield 4 fractions (Fr.EF4-1–EF4-4) and give a compound 2 (18.9 mg). Fr.EF4-2-3 (169 mg) was purified by SiO2 column and eluted by PE-EtOAc (20:1–0:1) to produce a compound 5 (28.8 mg). Normal phase silica gel column chromatography was performed on Fr.EH. After using PE-EtOAc (15:1–0:1) to perform gradient elution, and then Fr.EH2 was eluted with PE-EtOAc (25:1–0:1) to obtain a compound 3 (166.7 mg). The PE extract (38.56 g) was used on SiO2 CC and fully eluted with PE-EtOAc (100:1–0:1) to obtain 6 fractions (Fr.PA–PF). Fr.PC (11 g) was purified by SiO2 column using PE-EtOAc (100:1–0:1) to yield 3 fractions (Fr.PC1–PC3). Fr.PC2 was purified through SiO2 column and eluted with PE-CH2Cl2 (100:1–0:1) to give a compound 4 (30 mg).

Alisol B 23-acetate (1): White amorphous powder; TOF-MS m/z 537 [M+Na], C32H50O5 1H-NMR (300 MHz, CDCl3, δH): 4.59 (1H, m, H-23), 2.73 (1H, d, J = 8.5 Hz, H-24), 2.55 (1H, dd, J = 13.2, 5.7 Hz, H-12), 2.07 (3H, s, OAc), 1.71 (1H, d, J = 10.7 Hz, H-9), 1.33, 1.31, 1.14, 1.07, 1.05, 1.03, 0.96 (3H, each, s), 1.02 (3H, d, J = 7.1 Hz, H-21); 13C-NMR (75 MHz, CDCl3, δc): in Table 1.

Alisol C 23-acetate (2): White amorphous powder; TOF-MS m/z 551 [M+Na], C32H48O6 1H-NMR (300 MHz, CDCl3, δH): 4.55 (1H, m, H-23), 2.42 (1H, m, H-15β), 2.07 (3H, s, OAc), 1.88 (1H, m, H-15α), 1.75 (1H, m, H-22α), 1.19 (3H, d, J = 7.0 Hz, H-21), 1.32, 1.29, 1.22, 1.09, 1.08, 1.07, 0.89 (3H, each, s); 13C-NMR (75 MHz, CDCl3, δc): in Table 1.

**Table 1** 13C-NMR spectroscopic data of compounds 1–5 (δ in ppm)

| 1   | 2   | 3   | 4   | 5   |
|-----|-----|-----|-----|-----|
| C-1 | 30.9| 30.8| 31.0| 55.0| 50.6|
| C-2 | 36.7| 33.6| 33.8| 24.8| 22.5|
| C-3 | 220.1| 219.4| 220.1| 40.2| 40.4|
| C-4 | 47.0| 47.0| 47.0| 80.7| 80.2|
| C-5 | 48.4| 48.7| 48.5| 47.3| 50.3|
| C-6 | 20.1| 20.0| 20.1| 121.3| 121.3|
| C-7 | 34.2| 34.8| 34.3| 149.8| 149.7|
| C-8 | 40.7| 40.0| 40.6| 30.0| 25.1|
| C-9 | 49.9| 49.8| 49.6| 37.1| 42.5|
| C-10| 36.9| 36.9| 36.9| 153.9| 75.3|
| C-11| 70.1| 69.8| 69.8| 37.4| 37.3|
| C-12| 34.4| 35.5| 34.3| 21.3| 21.2|
| C-13| 138.2| 177.2| 138.1| 21.5| 21.4|
| C-14| 57.0| 49.8| 57.0| 24.1| 21.5|
| C-15| 30.6| 45.7| 30.6| 106.5| 21.3|
| C-16| 29.1| 208.1| 29.1|  |  |
| C-17| 134.1| 134.4| 134.8|  |  |
| C-18| 23.2| 23.1| 23.3|  |  |
| C-19| 25.7| 25.5| 25.5|  |  |
| C-20| 27.8| 26.7| 27.7|  |  |
| C-21| 20.1| 20.1| 20.2|  |  |
| C-22| 33.7| 35.0| 38.8|  |  |
| C-23| 71.5| 71.9| 69.1|  |  |
| C-24| 65.1| 64.9| 67.8|  |  |
| C-25| 58.5| 58.7| 59.2|  |  |
| C-26| 19.4| 19.7| 19.2|  |  |
| C-27| 24.7| 24.7| 24.9|  |  |
| C-28| 29.6| 29.6| 29.6|  |  |
| C-29| 20.0| 19.3| 20.1|  |  |
| C-30| 23.8| 23.1| 24.0|  |  |

1–5 in CDCl3, 75 MHz
22β), 2.80 (1H, dd, J = 13.4, 5.7 Hz, H-12α), 2.69 (1H, d, J = 8.1 Hz, H-24), 1.73 (1H, d, J = 10.7 Hz, H-9), 1.50 (1H, m, H-22α), 1.02 (3H, d, J = 7.0 Hz, H-21), 1.30, 1.22, 1.11, 1.07, 1.05, 1.04, 0.98 (3H, each, s); 13C-NMR (75 MHz, CDCl₃, δc); in Table 1.

Alismol (4): Colorless oil; TOF-MS m/z 220, C₁₅H₂₄O; 1H-NMR (300 MHz, CDCl₃, δH): 5.57 (1H, s, H-6), 4.72, 4.78 (1H, each, s, H₆-15), 1.26 (3H, s, H-14), 1.01 (3H, d, J = 6.8 Hz, H-13), 0.99 (3H, d, J = 6.8 Hz, H-12); 13C-NMR (75 MHz, CDCl₃, δc); in Table 1.

Alismoxide (5): White amorphous powder; EI-MS m/z 220 [M-H₂O]+, C₁₅H₂₉O₂; 1H-NMR (300 MHz, CDCl₃, δH): 5.50 (1H, d, J = 2.6 Hz, H-6), 1.27 (3H, s, H-15), 1.21 (3H, s, H-14); 1.01 (3H, d, J = 6.7 Hz, H-13), 0.98 (3H, d, J = 6.7 Hz, H-12); 13C-NMR (75 MHz, CDCl₃, δc); in Table 1.

### Antimicrobial susceptibility test

The antibacterial test was performed using six strains of bacteria. The Culture Collection of Antimicrobial Resistant Microbes of Korea (CCARM) supplied three drug-resistant bacteria strains of methicillin-resistant Staphylococcus aureus (MRSA CCARM 3506) and quinolone-resistant Staphylococcus aureus (QRSA CCARM 3505 and CCARM 3519). The Korean Collection for Type Cultures (KCTC) supplied the remaining bacteria strains including two G⁺ bacteria of Streptococcus mutans (KCTC 3289) and Staphylococcus aureus (KCTC 209), one G⁻ bacteria of Escherichia coli (KCTC 1924). The bacteria were cultured in Mueller–Hinton Broth (MHB) and grown to mid-log phase, and then diluted 1000 times in the medium under the same environment. Under aseptic conditions, the cells were inoculated into MHB broth, and then evenly distributed into 96-well microtiter plates for further cultivation. The antibacterial activity of the compounds was tested by serial dilution to determine the minimum inhibitory concentration (MICs). A microtiter ELISA reader was used to measure the absorbance at 650 nm to determine bacterial growth.

### Results and discussion

Three tetracyclic triterpenoids (1–3) and two sesquiterpenes (4, 5) were isolated from the methanolic extract of A. rhizoma. Their chemical structures were determined as alisol B 23-acetate (1), alisol C 23-acetate (2), alisol B (3), alismol (4), alismoxide (5) (Fig. 1) using spectroscopic methods including 1D, 2D NMR (HMBC, HMQC) and TOF-MS spectra.

Compound 1, white amorphous powder. Its molecular weight was determined to be 514 from the molecular ion peak at m/z 537 [M+Na]+ in TOF-MS. The 1H-NMR spectrum showed three oxygenated methine proton signals at δH 4.59 (m, H-23), 3.80 (m, H-11) and 2.73 (d, J = 8.5 Hz, H-24), one acetyl group at δ 2.07 (3H, s), seven tertiary methyl groups at δ 0.96, 1.03, 1.05, 1.07, 1.14, 1.31, 1.33 (each 3H, s) and one secondary methyl proton signal at δH 1.02 (d, J = 7.1 Hz). The 13C-NMR spectral data revealed 32 carbons, including one ketone carbon (δc 220.1), one carbonyl carbon (δc 170.1), two quaternary olefinic carbons (δc 138.2 and 134.1), three oxygenated methine carbons (δc 65.1, 70.1, 71.5), one oxygenated quaternary carbon (δc 58.5), seven tertiary methyl carbons (δc 19.4, 20.0, 23.2, 23.8, 24.7, 25.7, 29.6), one secondary methyl carbon (δc 20.1) and one acetyl methyl carbon (δc 21.2). Thus, compound 1 was identified to be alisol B 23-acetate by comparison with those found in the literature [14].

Compound 2 showed very similar 13C-NMR spectrum with that of 1, except for one carbonyl carbon at δc 208.1 (C-16) in place of one methylene carbon. In comparison with chemical shifts of C-13, C-14 and C-15, downfield shift at C-13 (δc 177.2) and C-15 (δc 45.7), upfield shift at C-14 (δc 49.8) were observed in 2. Compound 2 was determined to be alisol C 23-acetate by comparison with previously reported data [14].

Compound 3 showed very similar NMR signals with that of 1, except that acetyl group of C-23 was converted to hydroxyl group in 3. By comparison with previously reported data, 3 was identified as alisol B [14].

Compound 4, colorless oil. The molecular formula was determined to be C₁₅H₂₄O on the basis of a TOF-MS peak at m/z 220 [M]. By analyzing the carbon spectrum data, compound 4 was indicated that it was a sesquiterpene skeleton. The 1H-NMR spectrum showed one olefinic methine proton signal at δH 5.57 (1H, s, H-6), a couple of exomethylene proton signals at δH 4.78 and 4.72 (1H, each, s, H₆-15), and three methyl proton signals at δH 1.26 (3H, s, H-14), δ 1.01 (3H, d, J = 6.8 Hz, H-13) and 0.99 (3H, d, J = 6.8 Hz, H-12). The 13C-NMR spectrum confirmed the presence of 15 carbon signals, including four olefinic carbons at δc 153.9 (C-10), 149.8 (C-7), 121.3 (C-6) and 106.5 (C-15), one oxygenated carbon at δc 80.7 (C-4), three methine carbons at δc 55.0 (C-1), 47.3 (C-15) and 37.4 (C-11), along with three methyls at δc 24.1 (C-14), 21.5 (C-13) and 21.3 (C-12). From the NMR spectroscopic data, suggested that 4 is bicyclic sesquiterpenes, which is derivative of pseudoguaiane-type sesquiterpene. Thus, the structure of 4 was identified as alismoxide [15].

The molecular weight of 5 was determined to be 238 from the molecular ion peak at m/z 220 [M-H₂O]+ in EI-MS, indicating that the structure of 5 had one more hydroxyl group than 4. NMR signals of 5 were similar to those of 4, with the exception of the signals from exomethylene double bond of 4 converted into methyl group (δH 1.27, δc 21.3) and tertiary alcohol group (δc 75.3) in 5. Consequently, compound 5 was determined as alismoxide [15].
Antibacterial activity
All isolated compounds were evaluated the antibacterial potential against six strains of bacteria including three drug-resistant bacteria (MRSA CCARM 3506, QRSA CCARM 3505 and CCARM 3519), two $G^+$ bacteria ($S. mutans$ KCTC 3289 and $S. mutans$ KCTC 209) and one $G^-$ bacteria ($E. coli$ KCTC 1924). All compounds displayed strong antibacterial effect against $S. mutans$ 3289 with the MIC values of 2–64 µg/mL (Table 2). Among them, compound 1 had the strongest antibacterial activity, even more than streptomycin (MIC: 8 µg/mL), positive control. In particular, when acetyl group of compound 1 was converted to hydroxyl group in 3, the MIC value has increased from 2 to 16 µg/mL; methylene group at C-16 of 1 was converted to ketone group in 2, the MIC value has also increased from 2 to 64 µg/mL.

Table 2 The MICs of five compounds isolated from $A. rhizoma$ (µg/mL)

| Bacteria            | 1  | 2  | 3  | 4  | 5  | Chloramphenicol | Streptomycin |
|---------------------|----|----|----|----|----|----------------|--------------|
| $S. aureus$ CCARM 3519 | > 256 | > 256 | > 256 | 128 | > 256 | 8              | 16           |
| $S. aureus$ CCARM 3506 | > 256 | > 256 | 128 | 128 | > 256 | 8              | 8            |
| $S. aureus$ CCARM 3505 | > 256 | > 256 | > 256 | 128 | > 256 | 8              | 16           |
| $S. mutans$ KCTC 3289 | 2  | 64 | 16 | 32 | 32 | 1              | 8            |
| $S. aureus$ KCTC 209  | > 256 | > 256 | > 256 | 128 | > 256 | 8              | > 64         |
| $E. coli$ KCTC 1924   | > 256 | > 256 | > 256 | 128 | > 256 | 4              | > 64         |
mL. This observation suggested that the absence of acetyl group at C-23 or the presence of ketone group at C-16 in protostane-type resulted in the loss of antibacterial activity against S. mutans 3289. In addition, compounds 4 and 5 exhibited the same antibacterial activity with MICs of 32 μg/mL, indicating that the presence of exomethylene group at C-10 of guaiane-type sesquiterpene had not effect on antibacterial activity. Moreover, the minimum bacterial concentration (MBC) values of five compounds were lower than 4×MIC (Table 3).

In conclusion, five compounds including three triterpenoids and two sesquiterpenes were isolated from A. rhizoma. All compounds showed strong antibacterial activities against S. mutans 3289 with MIC values of 2–64 μg/mL. Among them, compound 1 had a good potential for use antibacterial agents. In addition, from the structure effective relationship, it found that acetyl group at C-23 and/or ketone group at C-16 in protostane-type is probably active group. It is the first report on antibacterial activity of all isolated compounds against six bacteria strains. Taken together, these results could provide potential sources of antibacterial compounds for A. rhizoma.

Acknowledgements
This research was supported by Natural Science Foundation of Jilin Province (ProjectNO.: 20200201149JC), People’s Republic of China.

Authors’ contributions
CF Li analyzed data and wrote the original draft. W Yan analyzed the antibacterial activity. EJ Cui reviewed and edited the manuscript. CJ Zheng administered this study. All authors read and approved the final manuscript.

Funding
Funding received from Department of Science and Technology of Jilin Province, People’s Republic of China.

Availability of data and materials
All data analyzed during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

Received: 6 November 2020 Accepted: 15 December 2020 Published: 19 January 2021

Table 3 The MBCs of five compounds isolated from A. rhizoma (μg/mL)

| Bacteria       | 1    | 2    | 3    | 4    | 5    | Chloramphenicol | Streptomycin |
|---------------|------|------|------|------|------|-----------------|-------------|
| S. aureus CCARM 3519 | > 256 | > 256 | > 256 | 256  | > 256 | 8               | 16          |
| S. aureus CCARM 3506 | > 256 | > 256 | 256  | 256  | > 256 | 8               | 8           |
| S. aureus CCARM 3505 | > 256 | > 256 | > 256 | 256  | > 256 | 8               | 8           |
| S. mutans KCTC 3289 | 2    | 64   | 16   | 32   | 32   | 1               | 8           |
| S. aureus CCARM 209 | > 256 | > 256 | > 256 | 256  | > 256 | 8               | 16          |
| E. coli KCTC 1924 | > 256 | > 256 | > 256 | 256  | > 256 | 8               | 8           |

References
1. Martens E, Demain AL (2017) The antibiotic resistance crisis, with a focus on the United States. J Antimicrob Chemother 72:502–526
2. Liu SS, Guo J, Li ZA, Tian SS, Zhu JJ, Yan LH, Wang ZM, Gao L (2020) Advances in studies on chemical compositions of Alismatis Rhizoma and their biological activities. China J Chin Mater Med 45:1578–1595
3. Jang MK, Han YR, Nam JS, Han CH, Kim BJ, Jeong HS, Ha KT, Jung MH (2015) Protective effects of Alisma orientale extract against hepatic steatosis via inhibition of endoplasmic reticulum stress. Int J Mol Sci 16:26151–26165
4. Dou F, Miao H, Wang JW, Chen L, Wang M, Chen H, Wen AD, Zhao YY (2018) An integrated lipidomics and phenotype study reveals protective effect and biochemical mechanism of traditionally used Alisma orientale Juzepzuk in chronic kidney disease. Front Pharmacol 9:53–70
5. Kubo M, Matsuda H, Tomohiro N, Yoshikawa M (1997) Studies on Alismatis rhizoma. I. Anti-allergic effects of methanol extract and six terpene components from Alismatis rhizoma (dried rhizome of Alisma orientale). Biol Pharm Bull 20:511–516
6. Kim Kh, Song HH, Ahn KS, Oh SR, Sadikot RT (2016) Ethanol extract of the tuber of Alisma orientale reduces the pathologic features in a chronic obstructive pulmonary disease mouse model. J Ethnopharmacol 188:21–30
7. Han CW, Kang ES, Ham SA, Woo HJ, Lee JH, Seo HG (2012) Antioxidative effects of Alisma orientale extract in palmitate-induced cellular injury. Pharm Biol 50:1281–1288
8. Jeong HS, Cho YH, Kim KH, Kim Y, Kim KS, Na YC, Park J, Lee IS, Lee JH, Jang HJ (2016) Anti-lipoapoptotic effects of Alisma orientalis extract on non-esterified fatty acid-induced HepG2 cells. BMC Complement Altern Med 16:239–250
9. Chen DQ, Feng YL, Tian T, Chen H, Yin L, Zhao YY (2014) Diuretic and anti-diuretic activities of fractions of Alismatis rhizoma. J Ethnopharmacol 157:114–118
10. Makino B, Kobayashi M, Kimura K, Ishimatsu M, Sakakibara I, Higuchi M, Kubo M, Sasaki H, Okada M (2002) Local variation in the content of angiotensin II and arginine vasopressin receptor antagonistic terpenoids in the rhizomes of Alisma orientale. Planta Med 68:226–231
11. Wang C, Zhang JX, Shen XL, Fan CH, Tse KW, Fong WF (2004) Reversal of P-glycoprotein-mediated multidrug resistance by salisal B 23-acetate. Biochem Pharmacol 68:635–659
12. Hyuga S, Shiraishi M, Hori A, Hyuga M, Hanawa T (2012) Effects of Kampo medicines on MDR-1-mediated multidrug resistance in human hepatocellular carcinoma HuH-7/PTX cells. Biol Pharm Bull 35:1729–1739
13. Xu GJ, Li JH, Yan W, Liu GJ, Cui EJ, Zheng CJ (2018) Antibacterial constituents from Magnolia officinalis. Lat Am J Pharm 37:1844–1849
14. Jin HG, Jin Q, Kim AR, Choi H, Lee JH, Kim YS, Lee DG, Woo ER (2012) A new triterpenoid from Alisma orientale and their antibacterial effect. Arch Pharm Res 35:1919–1926
15. Mona E, Namrita L, Ahmed H, Debra M (2013) Cytotoxic, cytotactic and HIV-1 PR inhibitory activities of the soft coral Litophyton arboreum. Mar Drugs 11:4917–4936

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.