Antibacterial compounds from *Atractylodes japonica* against Methicillin-resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging worldwide as one of the most important problems in dental and fields. Therefore, new agents are needed to treat MRSA from acute oral infection. In this study, antibacterial compounds from the roots of *Atractylodes japonica* (*A. japonica*) were isolated and characterized. Compounds were isolated from the roots extracts using HPLC-piloted activity-guided fractionations. Four compounds were isolated from the AJ and were identified as **1**, (6*E*, 12*E*)-teradecadiene-8,10-diyne-1,3-diol (TDEA); **2**, (6*E*, 12*E*)-teradecadiene-8,10-diyne-1,3-diol diacetate (TDEYA); **3**, atractylenolide I; and **4**, atractylenolide III. The minimum inhibitory concentrations (MICs) was determined in the setting of clinical MRSA isolates. Compound 1 showed anti-MRSA activity with MIC/MBC of was inhibited 4/32-8/63 mg/mL. The overall results provide promising baseline information for the potential use of the extract AJ as well as some of the isolated compounds in the treatment of bacterial infections.

Introduction

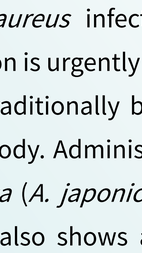
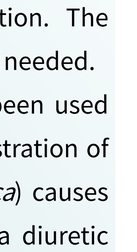
Methicillin-resistant *Staphylococcus aureus* (MRSA) has been accounts for a large proportion of hospital-acquired infections and is considered a serious problem because of its multi-drug resistant properties. Currently, vancomycin and its analog teicoplanin are the most effective antibiotics for MRSA infection. However, their clinical use often results in unexpected side effects and the development of vancomycin-resistant *S. aureus* infection. The search for better drugs to combat this infection is urgently needed.

Atractylodes japonica (*Compositae*) has traditionally been used for the treatment of water retention in the body. Administration of the aqueous extract of *Atractylodes japonica* (*A. japonica*) causes dieresis in humans and its alcohol extract also shows a diuretic effect in mice. *A. japonica* is known to be effective for the control of pain and treatment of arthritis. It was reported that the family of *Atractylodes* possesses anti-inflammatory activity and modulates the intestinal immune system. However, little is known about the antibacterial effects of *A. japonica* on MRSA. In the course of our ongoing project on the detection of bioactive compound from medicinal plant, the $CHCl_3$-soluble extract of roots of *A. japonica* was found to exhibit distinctive antibacterial activity.

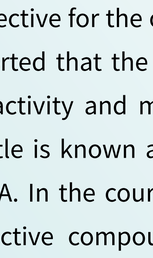
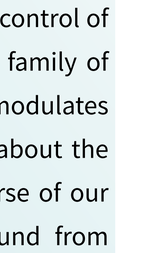
Materials and Methods

(1) Plant material, extraction and isolation

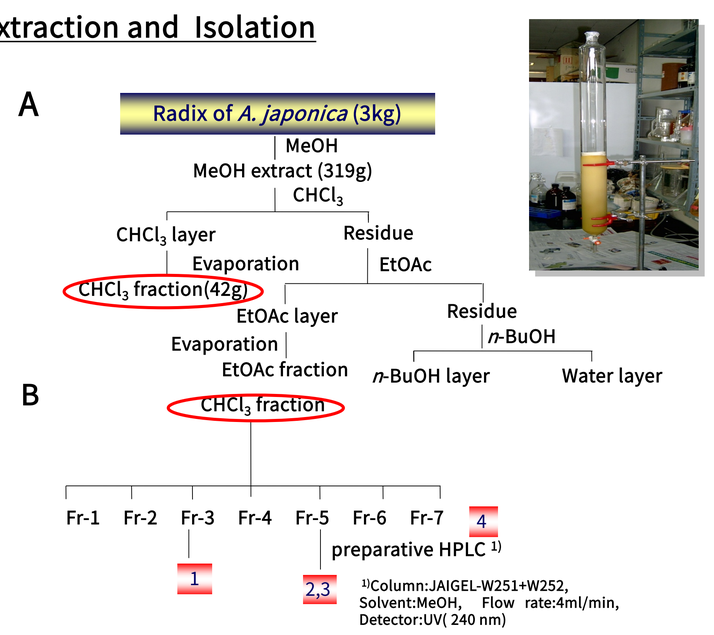
Parts used : Roots.
It is widely distributed in Korea, China and Japan.

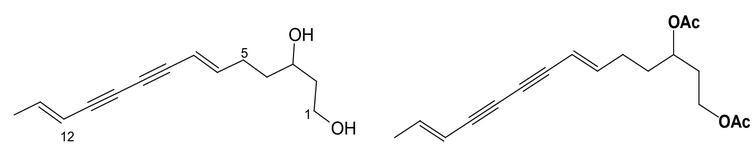



Medical fields :
A diuretic effect the control of pain and treatment of arthritis.
Modulates the intestinal immune system inflammatory diseases.

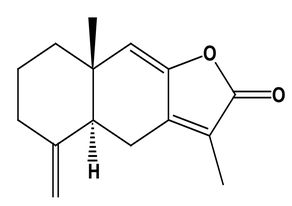
Extraction and Isolation

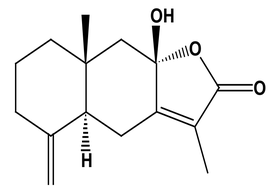




(6*E*, 12*E*)-teradecadiene-8,10-diyne -1,3-diol (TDEA) (1)

(6*E*, 12*E*)-teradecadiene-8,10-diyne -1,3-diol diacetate (TDEYA) (2)





Atractylenolide I (3)

Atractylenolide III(4)

Fig. 1. Chemical structures of isolated compounds from roots of *A. japonica.*

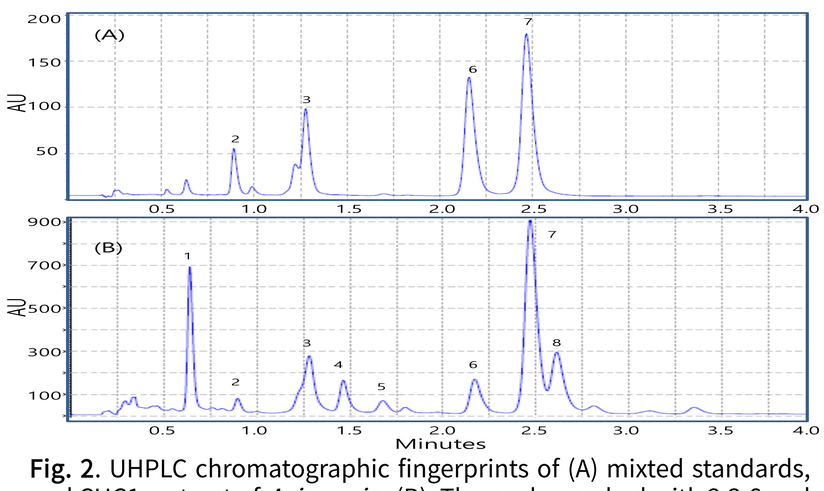


Fig. 2. UHPLC chromatographic fingerprints of (A) mixted standards, and $CHC1_3$ extract of *A. japonica* (B). The peaks marked with 2,3,6 and 7 are Atractylenolide III, TDEA, Atractylenolide I, and TDEYA, respectively. UHPLC condition; LC800 system, column; Inertsil ODS-4 (2μm, 50×2.1 mm I.D.), Flow rate; 400 μL/min, Inj. Vol.; 3μL, Detection; UV 240 nm, Eluent; H_2O:CH_3CN(34:66 (v/v)).

(2) Preparation of bacterial cells

Bacterial strains. The 14 MRSA isolates used in this study were obtained from the Wonkwang University Hospital (Iksan, Korea) and the standard strain of methicillin-resistant *Staphylococcus aureus* ATCC 33591 and *S. aureus* ATCC 25923 which is a MSSA (methicillin-susceptible *Staphylococcus aureus*). The MRSA strains were defined on the basis of the occurrence of the *mecA* gene and of their resistance to ampicillin and oxacillin, according to the guidelines of the Clinical Laboratory and Standard Institute (CLSI, 2009).

(4) Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of samples were determined by the broth dilution method and were carried out in triplicate. MICs were determined as the lowest concentration of test samples that resulted in a inhibition of visible growth in the broth. Following anaerobic incubation of MIC plates, the MBC were determined on the basis of the lowest concentration of the samples that kills of the test bacteria by plating out onto each appropriate agar plate. The agar plate containing only DMSO served as a control.

Results

Table 1. Antibacterial activities of methanol extract and different solvent fractions from of the roots of *A. japonica* against *S. aureus* ATCC 25923

Samples	MIC	MBC
MeOH extract	64	128
$CHCl_3$ fraction	32	64
EtOAc fraction	64	256
n-BuOH fraction	128	256

MIC and MBC, μg/mL

Table 2. Antibacterial activities of compound 1,2,3 and 4 isolated from the roots of *A. japonica* against *S. aureus* Korea isolate of 12 MRSA, standard MSSA and MRSA strains

		MIC/MBC (μg/mL)							
	Class	1		2		3		4	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 25923	MSSA	16	32	64	256	64	64	32	64
S. aureus ATCC 33591	MRSA	16	32	256	512	64	128	64	128
S. aureus 78	MRSA	8	32	64	512	64	128	32	32
S. aureus M11	MRSA	8	16	64	512	128	256	16	64
S. aureus 21-8	MRSA	4	8	32	128	128	128	64	128
S. aureus 6-2	MRSA	8	16	64	512	64	512	64	516
S. aureus 7-3	MRSA	32	64	64	512	64	128	128	128
S. aureus 8-4	MRSA	16	32	128	128	64	64	64	128
S. aureus 9-5	MRSA	16	64	128	512	128	256	32	64
S. aureus 13-7	MRSA	4	32	32	128	128	512	32	128
S. aureus 27-9	MRSA	8	16	512	512	64	512	64	128
S. aureus 47-10	MRSA	16	32	32	64	64	128	64	256
S. aureus 105-13	MRSA	4	8	8	64	64	128	32	128
S. aureus 106-14	MRSA	4	8	16	32	32	64	32	128

Conclusions

Bioassay-guided isolation of a chloroform extract of *Atractylodes japonica* (*Compositae*) led to the characterization of the 1, (6*E*, 12*E*)-teradecadiene-8,10-diyne-1,3-diol (TDEA); **2**, (6*E*, 12*E*)-teradecadiene-8,10-diyne-1,3-diol diacetate (TDEYA); **3**, atractylenolide I; and **4**, atractylenolide III. as the major anti-staphylococcal principle. MIC/MBC of compound 1 values ranged from 4/4–32/64μg/mL against methicillin-resistant *Staphylococcus aureus* strains.