Isolation of Antifungal Compound Against *Phytophthora infestans* from *Stellera chamaejasme* L.

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Our present investigation was in order to isolate antifungal constituents from the roots of *Stellera chamaejasme* L., using activity against several phytopathogenic fungi as a lead. Bioactivity guided fractionation of the ethyl acetate extract has led to the isolation of a novel antifungal compound (compound 1), which has exhibited a potent antifungal activity against *Phytophthora infestans* (EC₅₀ 138 mg/L). The antimicrobial activity of this compound against *Phytophthora infestans* has never been studied before. Its structure was established as chamaechromone on the basis of spectral analysis data, including ¹H NMR, ¹³C NMR and high resolution mass spectrometry.

Key Words: *Stellera chamaejasme* L., Chamaechromone, *Phytophthora infestans*, Antifungal activity.

INTRODUCTION

Stellera chamaejasme L. is a traditional Chinese medicine distributed extensively over northwest of China. It is usually used as herbal remedy for scabies, tinea and tuberculosis. Recently it has been found to show obvious anti-tumor activity¹. The main chemical compositions of it are flavonoids, coumarin derivatives, etc.²⁻⁸. In previous research of biologically pesticides based on plants, *Stellera chamaejasme* L. has been found to possess obvious bioactive activities, especially insecticidal activity. Some insecticide-activity compounds have been separated from it, such as 1,5-diphenyl-1-pentanone, 1,5-diphenyl-2-penten-1-one, umbelliferone, daphnoritin, etc.⁹⁻¹⁴. We have now identified the major antifungal constituent against *Phytophthora infestans* and it turned to be compound 1 (Fig. 1). We also investigated the activity of *Stellera chamaejasme* L. against various phytopathogenic fungi (*Botrytis cinerea*, *Penicillium italicum*, *Helminthosporium carbonum*, *Phytophthora infestans*, *Sclerotinia sclerotiorum* de Bary., *Pestalotiopsis theae*, *Helminthosporium maydis* Nisik & Miy, *Thanatephorus cucumeris* (Frank) Donk) according to the standard operating procedure for new agrochemicals (SOP) method of China¹⁵.

EXPERIMENTAL

IR (KBr pellet) and UV (in MeOH/H₂O) were recorded on Shimadzu IR-460 and a Shimadzu SPD-M10AVP photodiode detector. ¹H and ¹³C NMR spectra were measured at 27 °C in CD₃COCD₃ on Bruker AV II-400 MHz and Bruker AV II-600 MHz nuclear magnetic resonance apparatuses. HR-MS was recorded on Bruker MS BioTOF-Q. HPLC was performed on a Shimadzu LC-2010, with a Diamonsil C₁₈ column.

Fig. 1. Structure of the compound 1

The roots of *Stellera chamaejasme* L., growing at 4200 m above sea-level, were collected from Ruo Ergai County, Sichuan Province, P.R. China. The specimen was identified by Prof. Taiping Hou of Sichuan University and deposited in the Institute for Pesticides and Crop Protection of Sichuan University.

Microorganisms: Microorganisms used for bioassays were: *Botrytis cinerea*, *Penicillium italicum*, *Helminthosporium carbonum*, *Phytophthora infestans*, *Sclerotinia sclerotiorum* de Bary., *Pestalotiopsis theae*, *Helminthosporium maydis* Nisik & Miy, *Thanatephorus cucumeris* (Frank) Donk. *Phytophthora infestans* was provided by the Jiangsu Academy of Agricultural Sciences. The others were available in the Key Laboratory of Bio-resources and Eco-environment, Ministry of Education.

Extraction and isolation: The air-dried, powdered roots of *Stellera chamaejasme* L. (300 g) were extracted with 95 %

EtOH (3000 mL) at room temperature and the solvent was evaporated *in vacuo* yielding a brown viscous extract (38.13 g). The crude extract was suspended in two times weight of white diatomite and then partitioned with petroleum ether (PE, 60-90 °C), ethyl acetate (EtOAc) and methanol (MeOH) successively, yielding PE extract (5.4 g), EtOAc extract (6.9 g) and MeOH extract (0.7 g) after removal of solvents. The EtOAc extract which showed the best antifungal activity in the antifungal bioassays was subjected to Si-gel CC using a stepwise gradient elution of CH₂Cl₂/EtOAc mixed solvents (1/1-1/8, v/v) and pure EtOAc to yield six fractions (A-F) on the basis of similar TLC profiles under 274 nm UV detection. Fraction D which showed antifungal activity was chromatographed on a silica gel column again and eluted with hexane: chloroform (1/1.5, 1/2) to give 2 compounds (**1**, **2**). The high antifungal compound **1** is purified by high performance liquid chromatography (chloroform-methanol, 60:40).

Antifungal bioassays: Antifungal activity were tested against several phytopathogenic fungi (*Botrytis cinerea*, *Penicillium italicum*, *Helminthosporium carbonum*, *Phytophthora infestans*, *Sclerotinia sclerotiorum* de Bary., *Pestalotiopsis theae*, *Helminthosporium maydis* Nisik and Miy) in PDA media at 28 °C or Rye medium (RM) media at 20 °C. The various fungi were first incubated at 28 °C or 20 °C for 72 h. The zones of growth were recorded after the control group had a growth cycle diameter *ca.* 40-60 mm. The results are reported in Table-2. The antifungal activity was determined by the plate growth rate method according to the SOP method. The zone of growth cycle was expressed as an average of the maximum diameter in four different directions. The antifungal activities were expressed as both the inhibitory rate at 4 mg/mL and EC₅₀ (the concentration inhibiting growth by 50 %). All assays were conducted in triplicate.

RESULTS AND DISCUSSION

The ethanol crude extract *Stellera chamaejasme* L. is strongly active against *Phytophthora infestans* and *Sclerotinia sclerotiorum* de Bary. (Table-1). The inhibitory rates are 90.2 % and 80.1 % respectively. The various soluble parts and fractions (A-F) of EtOAc soluble part showed different inhibition against *Phytophthora infestans*. The EC₅₀ values were between 151 mg/L and 2890 mg/L (Table-2). The compound **1** which was isolated from the EtOAc extract, exhibited a potent antifungal activity against *phytophthora infestans*. The EC₅₀ value was 138 mg/L.

Bioactivity guided fractionation of EtOAc extract of *Stellera chamaejasme* L. ultimately led to the isolation of a novel compound **1**, which was obtained as a brown amorphous powder at room temperature. It showed positive reaction in FeCl₃ reaction. The molecular formula was determined as C₃₀H₂₂O₁₀ by HRMS. The IR spectrum showed the presence of hydroxyl (3377 cm⁻¹) groups, carbon-carbon double bond (1630 cm⁻¹, 1511 cm⁻¹) and carbon-oxygen bond (1239 cm⁻¹, 1172 cm⁻¹). Data (Table-3) of ¹H NMR δ (in ppm) 6.64 (2 H, d, *J* = 8.4 Hz), 6.59 (2 H, d, *J* = 8.4 Hz), 7.13 (2 H, d, *J* = 8.4 Hz), 7.24 (2 H, d, *J* = 8.4 Hz) indicated the presence of *p*-substituted benzene ring in the molecule. ¹H NMR δ 8.16 (1 H, s), 6.29 (1 H, s), 6.17(1 H, s) are the proton signals of

3-substituted-5,7-dihydroxychromone. It revealed that the compound probably existed chromone fragment. ¹H NMR δ 4.72 (1 H, d, *J* = 11.4 Hz), 6.54 (1 H, d, *J* = 12.0 Hz) are the signals of two mutual coupling adjacent hydrogen. Two protons at 5.81 and its associated carbon at 95.2 (C-16, 18) in the ¹H and ¹³C NMR spectra revealed the 1-,2-,4-,6-substituted benzene skeleton. ¹³C NMR δ 129.7, 114.7, 114.7, 129.7 and δ 128.7, 114.8, 114.8, 128.7 are the 2-,3-5-,7-carbon signals of typical *p*-substituted Benzene Ring. ¹³C NMR δ 156.2, 121.1, 180.2 are the 2-, 3-4-carbon signals of 3-substituted 5,7-dihydroxychromone. In contrast to previous literature assignments, the compound was elucidated as chamaechromone.

TABLE-1
In vitro ANTIFUNGAL ACTIVITY OF ETHANOL
EXTRACT (4 mg/mL) FROM *Stellera chamaejasme* L.

Fungus	Inhibition (%)
<i>Botrytis cinerea</i>	28.6
<i>Penicillium italicum</i>	6.1
<i>Helminthosporium carbonum</i>	10.3
<i>Phytophthora infestans</i>	90.2
<i>Sclerotinia sclerotiorum</i> de Bary	80.1
<i>Pestalotiopsis theae</i>	25.4
<i>Helminthosporium maydis</i> Nisik and Miy	73.9
<i>Thanatephorus cucumeris</i> (Frank)Donk	-

TABLE-2
INHIBITION OF MYCELIAL GROWTH BY DIFFERENT
FRACTIONS TO *Phytophthora infestans*

Samples	EC ₅₀ (mg/L)	Sample	EC ₅₀ (mg/L)
Ethanol extract	579	Fraction B	303
Petroleum ether part	2890	Fraction C	189
Ethyl acetate part	348	Fraction D	151
Methanol part	2427	Fraction E	680
Fraction A	1029	Fraction F	654
Compound 1	138		

TABLE-3
¹H-NMR (600 MHz), ¹³C NMR (400 MHz) DATA
FOR COMPOUND **1** IN CD₃COCD₃

Position	¹ H (J Hz)	Position	¹³ C	Position	¹³ C
2	8.16 (1 H, s)	2	156.2	17	164.8
6	6.17 (1 H, s)	3	121.1	18	95.2
8	6.29 (1 H, s)	4	180.2	19	164.0
11	4.72 (1 H, d, <i>J</i> = 11.4 Hz)	5	162.3	20	134.8
12	6.54 (1 H, d, <i>J</i> = 12.0 Hz)	6	98.9	21	128.7
16	5.85 (1 H, s)	7	164.3	22	114.8
18	5.85 (1 H, s)	8	93.5	23	155.6
21	7.13 (1 H, d, <i>J</i> = 8.4 Hz)	9	157.7	24	114.8
22	6.59 (1 H, d, <i>J</i> = 8.4 Hz)	10	104.5	25	128.7
24	6.59 (1 H, d, <i>J</i> = 8.4 Hz)	11	47.6	26	133.9
25	7.13 (1 H, d, <i>J</i> = 8.4 Hz)	12	53.3	27	129.7
27	7.24 (1 H, d, <i>J</i> = 8.4 Hz)	13	203.4	28	114.7
28	6.64 (1 H, d, <i>J</i> = 8.4 Hz)	14	105.4	29	155.5
30	6.64 (1 H, d, <i>J</i> = 8.4 Hz)	15	164.0	30	114.7
31	7.24 (1 H, d, <i>J</i> = 8.4 Hz)	16	95.2	31	129.7

In this study, we find that the total ethanol extract of *Stellera chamaejasme* L. and its solvent parts showed high inhibitory activity against *Phytophthora infestans*. Some antifungal compounds may be mixed in these extracts. Using bioassay-guided procedures, a high antifungal agent against *Phytophthora infestans* was isolated and identified as

chamaechromone. However the study throughout focus on the highest bioactive part of *Stellera chamaejasme* L. The fraction B and C of ethyl acetate soluble part also inhibit the growth of *Phytophthora infestans*. There may be other high bioactive compounds. So we can find more active constituents against *Phytophthora infestans* from fraction B and C or another solvent parts of *Stellera chamaejasme* L. during the next research.

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