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Isolation and Insecticidal Activity of Farnesol from *Stellera chamaejasme*

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The root powder of *Stellera chamaejasme* was extracted with petroleum ether. The crude extract obtained was chromatographed with silica gel columns several times. One pure natural product was obtained. It was identified as 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (farnesol), which was isolated for the first time in the root of *Stellera chamaejasme*. The results of laboratory bioassay showed that it had good insecticidal activity against *Aphis craccivora* and *Leucania separata*. Its median lethal concentrations (LC_{50}) (24 h after treatment) were 20.2 and 15.2 mg L^{-1}, respectively. This is the first report of the insecticidal activity of farnesol against *Aphis craccivora* and *Leucania separata*.

Key Words: Farnesol, Identification, Insecticidal activity, Isolation, *Stellera chamaejasme*.

INTRODUCTION

Stellera chamaejasme L. (family Thymelaeaceae) is a perennial herb with toxicity. Its root is used as Langdu in traditional Chinese medicine. It has certain therapeutic effects on some diseases including leucocythemia and cancer of the stomach[1]. It also possesses insecticidal activity against some pests[2,3]. Therefore, *Stellera chamaejasme* has been widely studied and many substances have been isolated and identified from it. To date, the substances separated have comprised the following four categories: diterpenoids, coumarins, lignins and biflavonoids. Among them, the separated natural products with insecticidal activity include 2-isopropyl-5-methylphenol and 5-isopropyl-2-methylphenol (unpublished results), 1,5-diphenyl-1-pentanone, 1,5-diphenyl-2-penten-1-one, 3-hydroxy-1,5-diphenyl-1-pentanone, daphnoritin, chamaechromone and 7-hydroxycoumarin[4-9].

Aphids, including *Aphis craccivora* and *Aphis gossypii*, are important pests in agriculture. In recent years, they have developed resistance to conventional synthetic insecticides including pyrethroids[10,11]. Moreover, their scope of resistance continues to expand and now has included many new organophosphorate and carbamate insecticides. Likewise, *Leucania separata* is a crop pest with serious harm. Over the past decades, synthetic insecticides, such as dimethoate, omethoate and methomyl, have been used to control it. However, the development of its resistance to all these synthetic insecticides has reduced the efficacy of insecticide treatment[12]. Therefore, new pesticides are continually necessary.

Secondary metabolites of plants are thought to act as toxicants, attractants or deterrents[13-15]. Therefore, based on bioassay results, the extraction and isolation of plant materials are considered one of the most successful methods to discover new natural products with insecticidal activity. In the present study, the isolation, identification and insecticidal activity of farnesol in the root of *Stellera chamaejasme* are reported.

EXPERIMENTAL

Stellera chamaejasme was collected from the Ruoergai grassland in Sichuan Province (China) and its identity was confirmed by experts related. *Aphis craccivora* and *Leucania separata* were reared for generations in the laboratory. They were maintained at 22-24 ºC, 60 % relative humidity (RH) and 14:10 h light: dark photoperiod. Vigorous and apterous adult aphids and third-instar larvae of *Leucania separata* were used in the experiments.

Extraction and isolation: Farnesol in the root of *Stellera chamaejasme* was extracted and separated according to the following procedures. The root powder of *Stellera chamaejasme* was extracted with petroleum ether (60-90 ºC) in a Soxhlet apparatus. The solvent was evaporated. The crude extract obtained was chromatographed in a silica gel (silica gel 60, 40-63 μm) column (40 cm × 6 cm), eluted successively with petroleum ether (60-90 ºC) + ethyl acetate (5:1) and petroleum ether (60-90 ºC) + ethyl acetate (1:1). Seven active fractions were obtained. The fraction with the second highest activity (the one with the highest activity had been isolated before hand) was chromatographed in a silica gel (silica gel 60, 40-63 μm)

column (40 cm × 3 cm), eluted successively with ethyl acetate + methanol (8:1 v/v) and ethyl acetate + methanol (1:1). Five active fractions were obtained. The fraction with the highest activity was chromatographed in a reverse phase silica gel (Lichroprep® RP-18, 25-40 μm) column (30 cm × 2 cm) with methanol + water (8:1 v/v) as the eluting solvent. One pure compound was obtained. The crude extract and its following fractions were monitored by testing their insecticidal activity against *Aphis craccivora*.

Spectroscopic analysis: Infrared spectra were recorded on the Nicolet MX-1EFT-IR spectrophotometer. Nuclear magnetic resonance (NMR) (^{1}H and ^{13}C NMR) spectra were taken on the Bruker Avance 600 instrument using deuteron-dimethyl sulfoxide (DMSO-d_6) as the solvent. Gas chromatograph-mass spectra (GC-MS) were obtained on the Shimadzu GC-MS-QP2010 instrument. High resolution mass spectra (HRMS) were taken on the Bruker Daltonics BioTOF Q instrument.

Bioassay of *Aphis craccivora*: The isolated product and imidacloprid (purity 90 %) were dissolved in dimethyl sulfoxide (DMSO), respectively[16]. Tween-80 was added to them at the rate of 1 %, respectively. The two solutions were diluted into five different concentrations (200, 100, 50, 25 and 12.5 mg L^{-1}; 20, 10, 5, 2.5 and 1.3 mg L^{-1}) with distilled water, respectively. Broad bean leaves with *Aphis craccivora* were picked. Vigorous and apterous adult aphids (30-50 head) were left on leaves by selection. The leaves with aphids were dipped in the solutions for 5 s. The leaves were allowed to air-dry. The aphids on them were transferred into Petri dishes (15 cm) with fresh broad bean leaves. The Petri dishes were placed in the laboratory held at 22-24 ºC, 60 % relative humidity and 14:10 h light: dark photoperiod. Control groups were treated with the corresponding solutions without the separated product or imidacloprid. The experiment for each concentration was replicated three times. Mortality was recorded 24 h after treatment. Percentage mortality was corrected according to the formula of Abbott[17] and LC_{50} values were calculated with the statistics package for the social sciences (SPSS) based on probit analysis[18].

Bioassay of *Leucania separata*: The isolated product and β-cypermethrin (purity 95 %) were dissolved in DMSO, respectively[16]. Tween-80 was added to them at the rate of 1 %, respectively. The two solutions were diluted into five different concentrations (100, 50, 25, 12.5 and 6.3 mg L^{-1}; 10, 5, 2.5, 1.3 and 0.6 mg L^{-1}) with distilled water, respectively. Maize leaves were cut into strips (2 cm × 5 cm) and dipped in the solutions for 5 s. The leaves were allowed to air-dry and placed in Petri dishes (9 cm). Ten third-instar larvae of *Leucania separata* were put into one Petri dish. The Petri dishes were placed in the laboratory held at 22-24 ºC, 60 % RH and 14:10 h light: dark photoperiod. Control groups were treated with the corresponding solutions without the isolated product or β-cypermethrin. The experiment for each concentration was replicated three times. The following procedures were the same as for the bioassay of *Aphis craccivora*.

RESULTS AND DISCUSSION

Purification and identification: After the crude extract had been separated and purified several times with gel silica columns, one pure natural product was obtained. It was identified as 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (Fig. 1) by its IR, NMR (^{1}H and ^{13}C NMR), GC-MS and HRMS.

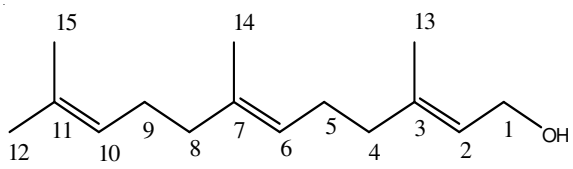

Fig. 1. Chemical structure of farnesol

The physico-chemical data of the isolated product ($C_{15}H_{26}O$) are as follows: colourless oily liquid; IR (liquid film, ν_{max}, cm^{-1}): 3322, 2964, 2920, 2856, 1666, 1445, 1375, 1000, 831;EI-MS (70 eV) m/z: 222 (M^+), 204, 137, 121, 109, 93, 81, 69, 55, 41, 27; HRMS: calcd. for $C_{15}H_{26}O$: 222.1977, found: 222.1978; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 3.91 (2H, m, H-1), 5.26 (1H, m, H-2), 1.91-2.06 (8H, m, H-4, 5, 8, 9), 5.08 (1H, m, H-6), 5.04 (1H, m, H-10), 1.65 (3H, s, H-12), 1.62 (3H, s, H-13), 1.56 (3H, s, H-14), 1.55 (3H, s, H-15), 4.37 (1H, m, OH); ^{13}C NMR (DMSO-d_6, 600 MHz) δ (ppm): 58.0 (1C, C-1), 124.5 (1C, C-2), 136.0 (1C, C-3), 39.7 (1C, C-4), 26.7 (1C, C-5), 124.3 (1C, C-6), 135.7 (1C, C-7), 39.5 (1C, C-8), 26.4 (1C, C-9), 124.2 (1C, C-10), 131.2 (1C, C-11), 23.5 (1C, C-12), 16.3 (1C, C-13), 16.1 (1C, C-14), 17.8 (1C, C-15).

Insecticidal activity against *Aphis craccivora*: The separated product was submitted to laboratorial bioassay compared with the super efficient insecticide imidacloprid. The results are presented in Table-1. It had good insecticidal activity *Aphis craccivora*. Its LC_{50} value was 20.2 mg L^{-1}. The results of regressive and correlative analysis indicated that the correlation was significant between concentration and efficacy. Its correlative coefficient was 0.9716. Chi square test demonstrated that the results were reliable ($\chi^2 = 2.925$, df = 3, $p > 0.05$).

TABLE-1
INSECTICIDAL ACTIVITY OF THE ISOLATED PRODUCT AGAINST *Aphis craccivora*

	Isolated product					Imidacloprid				
Concentration (mg L^{-1})	200	100	50	25	12.5	20	10	5	2.5	1.3
Mortality* (%)	95.2	80.5	71.3	55.0	39.6	99.2	87.1	74.7	58.2	43.1
Regressive equation (Y = aX + b)	Y = 1.4404X + 3.1204					Y = 1.6861X + 4.5873				
LC_{50} (mg L^{-1})	20.2					1.8				
(95 % CL)	(15.8-24.5)					(1.4-2.1)				
Correlative coefficient (r)	0.9716					0.9920				
χ^2	2.925					4.577				

*Based on the mean of triplicates corrected according to Abbott formula.

Insecticidal activity against *Leucania separata*: As shown in Table-2, the isolated product was subjected to laboratorial bioassay using the efficient insecticide β-cypermethrin as the comparative standard. Its LC_{50} value reached 15.2 mg L^{-1}. The results of regressive and correlative analysis revealed that the correlation was very significant between concentration and

TABLE-2
INSECTICIDAL ACTIVITY OF THE ISOLATED PRODUCT AGAINST *Leucania separata*

	Isolated product					β-Cypermethrin				
Concentration (mg L^{-1})	100	50	25	12.5	6.3	10	5.0	2.5	1.3	0.6
Mortality* (%)	93.3	76.7	60.0	40.0	33.3	96.7	83.3	60.0	43.3	33.3
Regressive equation (Y = aX + b)	Y = 1.5415X + 3.1793					Y = 1.7151X + 4.7584				
LC_{50} (mg L^{-1})	15.2					1.4				
(95 % CL)	(9.9-21.1)					(0.9-1.9)				
Correlative coefficient (r)	0.9830					0.9872				
χ^2	1.378					2.257				

*Based on the mean of triplicates corrected according to Abbott formula.

efficacy. The correlative coefficient was 0.9830. As for the results of *Aphis craccivora*, chi square test also showed that the results were reliable ($\chi^2 = 1.378$, df = 3, $p > 0.05$).

The results of laboratory bioassay have clearly demonstrated the insecticidal activity of the separated product against *Aphis craccivora* and *Leucania separata*. It is a known compound with many uses. It can be used as juvenile hormones and male sex attractants for insects[19,20]. It can also be used as fungicides[21]. But it is the first time that its insecticidal activity against the two different pests has been reported. Therefore, it may be a pest control agent with potential value. Moreover, if used as an insecticide, it will not bring about pollution and residues because it may degrade by itself in the environment. In addition, it was experimentally observed that the broad bean leaves and maize leaves did not display any abnormality after they had been dipped in the solutions. This showed that the isolated product might be harmless to them.

Although its insecticidal activity was inferior to the corresponding comparative standard, it has the advantages of botanical insecticides. What is more, since its structure is simple and its chemical synthesis is easy, its structure can be optimized and modified so as to discover new insecticides with high effect, low toxicity and safety to non-target organisms.

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