Acaricidal activity of extracts from *Ligularia virgaurea* against the *Sarcoptes scabiei* mite *in vitro*

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Abstract. The aim of the present study was to investigate the acaricidal activity of *Ligularia virgaurea*. An extract was prepared by refluxing with ethanol and steam distillation, and its toxic effect was tested *in vitro* against *Sarcoptes scabiei*. The data from the toxicity tests were analyzed using a complementary log-log (CLL) model. The ethanol extract exhibited strong acaricidal activity against these mites; at a concentration of 2 g/ml it killed all *S. scabiei* within 2 h and at 1 g/ml it killed all *S. scabiei* within 6 h. Similarly, 2, 1, 0.5 and 0.25 g/ml concentrations of the extract had strong toxicity against *S. scabiei*, with median lethal time (LT_{50}) values of 0.716, 1.741, 2.968 and 4.838 h, respectively. The median lethal concentration (LC_{50}) values were 1.388, 0.624, 0.310 and 0.213 g/ml for *Scabies* mite in 1, 2, 4 and 6 h, respectively. The results indicate that the *L. virgaurea* extract has strong acaricidal activity and may be exploited as a novel treatment for the effective control of acariasis in animals.

Introduction

Acariasis in animals, a common veterinary skin disease, may reduce the productivity and the quality of products obtained from animals, and can even lead to death (1). At present, various chemical agents are widely used to treat and control psoroptic and sarcoptic mange in veterinary clinics, and relatively good treatment effectiveness has been obtained with certain drugs, including ivermectin and abamectin. However, the use of chemicals could result in resistance in target species, toxicity and environmental hazards (2).

Ligularia Cass. of the family Compositae or Asteraceae is a genus highly diversified in the eastern Qinghai-Tibet Plateau region and adjacent areas, where >100 species exist (3,4). The *Ligularia* Cass. genus is one of the large genera in the Compositae-Senecioneae-Tussilagininae classification (5). *Ligularia virgaurea* is one of the main species of *Ligularia* Cass., and is a noxious weed widely distributed in the alpine grassland of the eastern Qinghai-Tibet Plateau (6). *Ligularia* Cass. has been used for medicinal purposes, particularly for the treatment of jaundice, scarlet fever, rheumatoid arthritis and hepatic function failure (7). Extracts of this genus of plants have been reported to have antioxidant (8), analgesic, anti-inflammatory (9) and antitumor activity (10); anti-ulcerogenic (11), anti-obesity (12) and antihepatotoxic effects (13) and anticomplement activity (14). However, to the best of our knowledge, the acaricidal activity of *L. virgaurea* has not been investigated. The aim of the present study was to evaluate the possible acaricidal activity of an ethanol extract of *L. virgaurea* against *Sarcoptes scabiei*.

Materials and methods

Plant material and extraction. L. virgaurea was collected from Ganzi in Sichuan Province, southwest China in July 2013. A thermal refluxing method was used to extract the biological components into ethanol, as previously described (15). Following the concentration of the extract by the evaporation of ethanol, the extract was diluted with distilled water containing glycerin (10%) to varying concentrations.

Mites. The *S. scabiei* mites were isolated from the scabs and ear cerumen of the infested legs and ears of naturally infected rabbits. The scabs and ear cerumen were placed in Petri dishes and incubated at 35˚C for 30 min in an incubator. The adult mites were then collected for testing. Following the collec-

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tion of the samples, the rabbits were treated immediately. Institutional ethical and animal care guidelines of South China Agricultural University Experimental Animal Ethics Committee were adhered to during the sampling exercise, and all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (8th ed, 2012).

In vitro assay of acaricidal activity. The ethanol extract was diluted to concentrations of 2, 1, 0.5 and 0.25 g/ml with 10% glycerin, and 0.5-ml samples of the extract were added to Petri dishes (diameter, 10 cm; height, 2 cm) with filter paper chips to absorb the liquid.

To test the activity against *S. scabiei*, 10 specimens were placed on the filter paper in the Petri dishes and incubated at 25˚C under 75% relative humidity for 0.5, 1, 2, 4 or 6 h (16,17). Three replicates were performed for each concentration of the extract. Three Petri dishes containing 0.5 ml distilled water and glycerin (16,18) acted as an untreated (negative) control, and 2% ivermectin was used as the positive control. The mites were regularly stimulated with a needle to determine their viability, and mites were recorded as dead if no reaction was shown (19).

Statistical analysis. All computations were conducted using SPSS statistical software (version 20.0; SPSS, Inc., Chicago, IL, USA) (20). The significance of differences in the mean mite mortality between different concentrations was calculated using a probability method. The median lethal time value (LT_{50}) and the median lethal concentration value (LC_{50}) were calculated by the complementary log-log (CLL) model (21).

Results

Considerable differences in acaricidal activity were observed among the different concentrations of the ethanol extract. The highest concentration of the extract (2 g/ml) caused 100% mortality in the test mites. In the negative control group, treated with 10% glycerin, the mites remained alive after the 6-h treatment period (Fig. 1). In addition, in the ivermectin-treated positive control group, a number of the mites remained alive in the sample following the 6-h treatment period. This may be because the *S. scabiei* test mites had developed resistance to ivermectin. Mortality rates for the mites treated with the four concentrations of the extract are shown in Table I. The toxicity of the ethanol extract was evaluated using a CLL model. Pearson's Chi-square test and the Hosmer-Lemeshow goodness-of-fit statistic indicated that the data fitted the CLL model. The LT_{50} and LC_{50} values of the ethanol extract against *S. scabiei* are shown in Tables II and III, respectively. The ethanol extract demonstrated strong toxicity to mites, and its activity was concentration- and time-dependent. The results indicate that an ethanol extract of *L. virgaurea* at concentrations of 2, 1 and 0.5 g/ml was highly toxic to *S. scabiei*. The LT_{50} values for solutions of the extract at concentrations of 2, 1, 0.5 and 0.25 g/ml were 0.716, 1.741, 2.968 and 4.838 h, respectively, and the LC_{50} values for 1, 2, 4 and 6 h treatment times were 1.388, 0.624, 0.310 and 0.213 g/ml, respectively.

Table I. Acaricidal activity of the ethanol extract of *Ligularia virgaurea* against *Sarcoptes scabiei in vitro*.

	Mean mortality, % ± SD				
Test agent	0.5 h	1 h	2 h	4 h	6 h
Extract					
2 g/ml	$36.67\pm6.67^{A,a}$	$66.67\pm8.82^{A,a}$	$100.00\pm0.00^{A,a}$	$100.00\pm0.00^{A,a}$	$100.00\pm0.00^{A,a}$
1 g/ml	$20.00\pm5.77^{A,a,c}$	$40.00\pm0.00^{B,b}$	$63.33\pm3.33^{B,b}$	$80.00\pm5.77^{B,b}$	$100.00\pm0.00^{A,a}$
0.5 g/ml	$13.33\pm6.67^{A,b,e}$	$30.00\pm5.77^{B,c}$	$46.67\pm8.82^{B,c,e}$	$63.33\pm3.33^{C,c}$	$80.00\pm5.77^{B,b}$
0.25 g/ml	$6.67\pm3.33^{A,b}$	$13.33\pm3.33^{C,d}$	$30.00\pm5.57^{B,d}$	$46.67\pm3.33^{D,d}$	$56.67\pm3.33^{C,c}$
Positive control	$10.00\pm5.77^{A,b}$	$16.67\pm3.33^{C,d}$	$36.67\pm3.33^{B,d,e}$	$66.67\pm3.33^{C,e}$	$83.33\pm3.33^{B,b}$
Negative control	$0.00\pm0.00^{B,c}$	$0.00\pm0.00^{D,e}$	$3.33\pm3.33^{C,f}$	$10.00\pm0.00^{D,d}$	$16.67\pm3.33^{D,d}$

$^{A-D}P<0.01$ between data with different capital letters within a column; $^{a-f}P<0.05$ between data with different lower case letters within a column.

Table II. Probit regression analysis of the toxicity (LT_{50}) of the ethanol extract of *Ligularia virgaurea* against *Sarcoptes scabiei in vitro*.

Concentration	Regression equation	LT_{50} (95% FL), h	Pearson Chi-square
2 g/ml	y=1.894x-1.357	0.716 (0.490-0.892)	0.521
1 g/ml	y=0.500x-0.871	1.741 (1.219-2.224)	3.606
0.5 g/ml	y=0.312x-0.926	2.968 (2.263-3.765)	2.567
0.25 g/ml	y=0.270x-1.306	4.838 (3.940-6.427)	2.753

LT_{50}, median lethal time; FL, fiducial limits.

Table III. Probit regression analysis of the toxicity (LC_{50}) of the ethanol extract of *Ligularia virgaurea* against *Sarcoptes scabiei in vitro*.

Time (h)	Regression equation	LC_{50} (95% FL), g/ml	Pearson Chi-square
1	y=0.777x-1.078	1.388 (1.076-1.922)	1.161
2	y=1.501x-0.936	0.624 (0.427-0.807)	1.770
4	y=1.423x-0.441	0.310 (0.058-0.504)	0.634
6	y=3.259x-0.694	0.213 (-0.092-0.322)	0.330

LC_{50}, median lethal concentration; FL, fiducial limits.

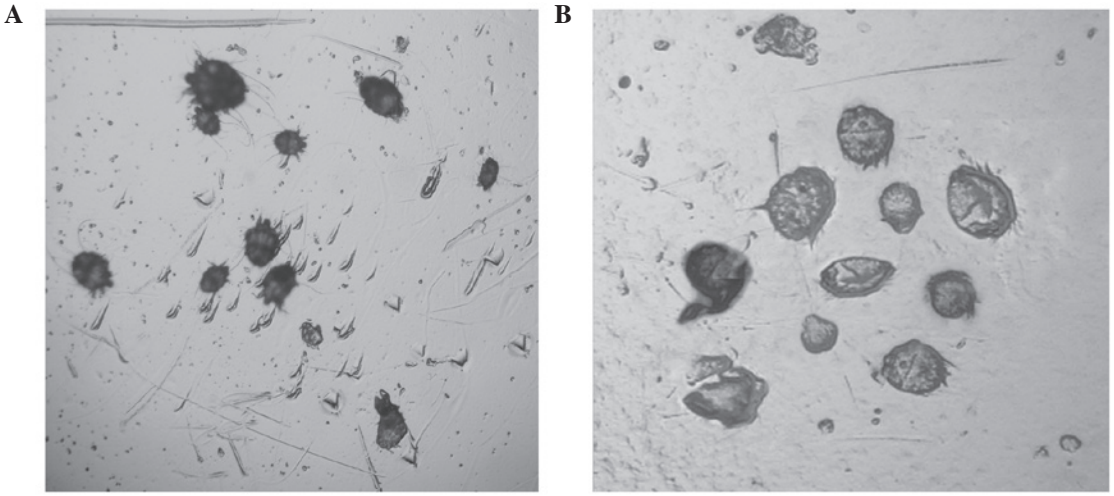

Figure 1. Samples of the mites after 6 h of treatment. (A) Negative control group (10% glycerin), two mites had died; (B) experimental control group (ethanol extract with 2 g/ml).

Discussion

The results of this study demonstrate for the first time, to the best of our knowledge, that an ethanol extract of *L. virgaurea* has strong acaricidal activity. The extract, obtained from *L. virgaurea* by refluxing in ethanol, exhibited a strong toxic effect against *S. scabiei*. The toxicity of the extract was shown to be time- and concentration-dependent. Similar effects have been observed for an ethanol extract of *Eupatorium adenophorum* against *S. scabiei* (17,22), 9-oxo-10,11-dehydroageraphorone extracted from *E. adenophorum* against *S. scabiei* and *Psoroptes cuniculi in vitro* (16,23), and for neem oil against *Amblyomma variegatum* (24). The results of the present study suggest that the ethanol extract from *L. virgaurea* contains acaricidal component(s). It is likely that the acaricidal component(s) are soluble in organic solvents, such as alcohol, methanol and acetone, and is consistent with previous observation that the acaricidal components obtained from natural plants are volatile compounds (25,26). Although *L. virgaurea* is regarded as a weed, this study provides a basis for its utilization in the development of plant-derived acaricidal drugs. These preliminary results indicate that *L. virgaurea* may be a source of novel acaricidal compounds that are able to effectively control mites in livestock. Further systematic studies are

warranted to identify the active compounds in *L. virgaurea* and to evaluate them in clinical trials, animal acute toxicity tests and safety tests. Previous studies have shown that some of the effective components from *L. virgaurea* are not toxic to humans or animals (27). Following further in-depth evaluation, *L. virgaurea*, as a potent herbal drug, may have the potential to be more widely applied in treatments for humans and animals.

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