

# Effect of acaricidal components isolated from lettuce (*Lactuca sativa*) on carmine spider mite (*Tetranychus cinnabarinus* Boisd.)

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## Abstract

This study aimed to evaluate the acaricidal activity of lettuce (*Lactuca sativa*) extracts against carmine spider mites (*Tetranychus cinnabarinus* Boisd.) and isolate the acaricidal components. Acaricidal activities of lettuce extracts isolated from different parts (the leaf, root and seed) using various solvents (petroleum ether, acetone and methanol) were evaluated with slide-dip bioassay and relatively high median lethal concentration (LC<sub>50</sub>) values were detected. Acetone extracts of lettuce leaves harvested in July and September were fractionated and isolated with silica gel and thin-layer chromatography. Consequently, acetone extracts of lettuce leaves harvested in July exhibited higher acaricidal activity than those harvested in September, with an LC<sub>50</sub> value of 0.268 mg ml<sup>-1</sup> at 72 h post-treatment. A total of 27 fractions were obtained from the acetone extract of lettuce leaves harvested in July, and mite mortalities with the 11th and 12th fractions were higher than those with the other 25 fractions (LC<sub>50</sub>: 0.751 and 1.258 mg ml<sup>-1</sup> at 48 h post-treatment, respectively). Subsequently, active acaricidal components of the 11th fraction were identified by infrared, nuclear magnetic resonance and liquid chromatography/mass spectrometry. Five components were isolated from the 11th fraction, with components 11-a and 11-b showing relatively high acaricidal activities (LC<sub>50</sub>: 0.288 and 0.114 mg ml<sup>-1</sup> at 48 h post-treatment, respectively). Component 11-a was identified as β-sitosterol. In conclusion, acetone extracts of lettuce leaves harvested in July might be used as a novel phyto-genic acaricide to control mites.

**Keywords:** *Lactuca sativa*, *Tetranychus cinnabarinus*, acetone, acaricidal activity, β-sitosterol

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## Introduction

Carmine spider mites (*Tetranychus cinnabarinus* Boisd.) of order Acarina and family Tetranychidae are one of the most

menacing polyphagous pests, causing significant harm to crops and vegetables in fields and greenhouses worldwide (Yu *et al.*, 2016). Mites can cause bronze or tan-coloured leaf stippling after infecting various plants, such as eggplants, capsciums, watermelons, beans and green onions (Schmidt, 2014). Currently, mites are mainly controlled by chemical synthetic acaricides, and the frequent application of simple chemical drugs always results in rapid development of drug resistance (Kwon *et al.*, 2014; Diaz, 2016). Furthermore, the use of chemical pesticides causes high mortalities of natural enemies and non-target organisms as well as undesirable effects on

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humans and the environment (Cavalcanti *et al.*, 2010; Nicolopouloustamati *et al.*, 2016). Therefore, it is crucial that novel, effective, anti-resistant, safe and eco-friendly chemical control alternatives are identified and developed.

Recently, phyto-genic acaricides that are non-toxic to mammals and have low to no residual effects on the environment were found to be reasonable candidates for mite management (Benelli *et al.*, 2016). Extracts of various plants such as *Polygonum aviculare*, *Stellera chamaejasme*, *Inula japonica*, *Juglans regia*, *Albizia julibrissin* and *Mentha piperita* show acaricidal activities against mites (Wang *et al.*, 2007b, 2013; Ren *et al.*, 2009a; Duan *et al.*, 2012). These phyto-genic acaricides have clear inhibitory effects on the growth and reproduction of mites through repellence, oviposition deterrence and growth regulation (Singh & Saratchandra, 2005).

Lettuce (*Lactuca sativa*), the most popular vegetable in salads (Oyinlola *et al.*, 2017), belongs to the botanical family of Asteraceae and is regarded as a healthy food due to the high contents of vitamin C, flavonols and fibre (Serafini *et al.*, 2002; Nicolle *et al.*, 2004; Llorach *et al.*, 2008). The medicinal values of lettuce have been revealed by various studies. In one such study, it was found that methanol extracts of lettuce exhibited a high hydroxyl radical-scavenging activity (IC 50 = 3.5 mg ml<sup>-1</sup>), low minimum inhibitory concentrations against Gram-positive bacteria (2.5 mg ml<sup>-1</sup>) and high anti-viral activity against HCMV and Cox-B3 viruses (IC 50 = 200 mg ml<sup>-1</sup>) (Edziri *et al.*, 2011). Extracts of lettuce seeds showed a dose-dependent anti-inflammatory activity in a carrageenan model of inflammation (Sayyah *et al.*, 2004). At 6–42 h post-treatment, the anti-feeding effect of lettuce extracts on *Phyllo-treta striolata* (F.) was 100% (Lai & You, 2004). However, studies on the acaricidal activity of lettuce extracts are still limited.

In this study, acaricidal activities of lettuce extracts of different plant parts (the leaf, root and seed) using various solvents (petroleum ether, acetone and methanol) were evaluated. Acetone extracts of lettuce leaves harvested in July and September were fractionated and isolated, and an active acaricidal component was identified. Our findings may reveal a novel phyto-genic acaricide without resistance or potential hazard to humans and the environment.

## Materials and methods

### Materials

Lettuce (Crisphead lettuce, Dongshan) seeds were purchased from a local market and identified by Professor Li X. Y. in the College of Horticulture, Southwest University. Lettuce seeds were sown in an experimental plot of Southwest University (Beipei, Chongqing, China) in April and June 2012, and leaf and root samples were harvested in July and September 2012. A *T. cinnabarinus* Boisid. colony was reared on cowpea plants (*Vigna unguiculata*) in a laboratory of Southwest University at 26 ± 1°C, 75–80% relative humidity (RH) and a photoperiod of 16:8 h (light:dark). These mites had been maintained for >16 years without exposure to any pesticides (Hou *et al.*, 2015). Petroleum ether, acetone, ethyl acetate, methanol and Tween-80 were purchased from Kelong Chemical Reagents Co., Ltd. (Chengdu, China).

### Extraction

Lettuce root, leaf and seed samples were dried at 50°C for 3 days and ground into powder and screened through an

80-mesh screen. A total of 50 g pretreated samples were immersed in 60–90°C petroleum ether, acetone or methanol at a proportion of 1.0 ml 0.2 g<sup>-1</sup> for 3 days at room temperature. After filtering, crude extracts were evaporated at 40°C using a vacuum rotary evaporator (R-201, Shanghai Shenshen) and kept in glass vials at 4°C until use (Ding *et al.*, 2013).

### Isolation of acaricidal components

Acetone extracts of lettuce leaves in July were purified by column chromatography (200–300 mesh, Haiyang Chemical Co. Ltd., Qingdao, China) and eluted with a gradient of petroleum ether:ethyl acetate (10:1, 8:1, 6:1, 4:1, 2:1, 0:1; v/v) and ethyl acetate:methanol (10:0, 5:1, 5:2, 5:3, 5:4, 1:1; v/v). A total of 138 initial fractions (75 ml each) were collected. Based on *R<sub>f</sub>* values, 27 fractions were obtained through thin-layer chromatography. Then, the fractions with high acaricidal activities (11th and 12th fractions) were further purified on a silica gel column (Haiyang Chemical Co. Ltd., Qingdao, China) and eluted with dichloromethane:ethyl acetate (200:1, v/v) (Ding *et al.*, 2013).

### Bioassays

A modified slide-dip method recommended by the Food and Agriculture Organization (FAO) was used to test the contact toxicity of different lettuce extracts against mites (Anonymous, 1980). A total of 40 adult female mites were fixed on a slide with double-sided adhesive tape using a fine brush and maintained at 26 ± 1°C and 65–80% RH for 4 h. Dead and inactive mites were removed after examination under a stereomicroscope (4×). Thirty remaining active mites were recorded as the starting number. Based on probit analysis in our previous study, 10 mg ml<sup>-1</sup> caused 95% corrected mortality and 0.313 mg ml<sup>-1</sup> caused 17% corrected mortality in carmine spider mites; therefore, concentrations 0.313, 0.625, 1.250, 2.50, 5.0 and 10.0 mg ml<sup>-1</sup> were chosen. Then, slides with mites were dipped into extract dilutions for 5 s. Each millilitre of extract solution contained 2.5 mg extract, 1% Tween-80 and 0.5% acetone (to increase solubility), and the control contained only the same percentage of Tween-80 and acetone. Propargite with 1% Tween-80 and 0.5% acetone was used as positive control. Excess solution on slides was removed using filter paper. Live and dead mites were counted at 24, 48 and 72 h post-treatment. A mite was considered to be dead when its legs or abdomen did not respond to repeated gentle probing with a fine brush. Corrected mortality was calculated using Abbott's formula recommended by FAO (Abbott, 1987):

$$\text{Corrected mortality} = \frac{(\text{Mortality}_{\text{Treatment group}} - \text{Mortality}_{\text{control group}})}{(1 - \text{Mortality}_{\text{control group}})} \times 100\%$$

Mite mortalities induced by the 27 fractions (1 mg ml<sup>-1</sup>) prepared from acetone extracts of lettuce leaves were also analysed. All treatments were tested under the same conditions as described above, and three replicates for each extract were performed.

Ovicidal activity of extracts was measured as previously described (Liu *et al.*, 2012). Mite eggs were numbered and placed in the centre of a Petri dish. After immersing in the diluted solution for 5 s, hatching rate was assessed at 120 h post-

treatment using the following formula:

$$\text{Ovicidal rate} = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} \times 100\%.$$

Finally, the median lethal concentration (LC<sub>50</sub>) value of extracts for eggs and adult mites was determined.

#### Component analysis

Components of lettuce extracts with high acaricidal activities were further identified by liquid chromatography/mass spectrometry (LC/MS) using a chromatograph (Water 2487-ZQ 4000) with an LC column (10 m, 2.1 mm, 3.5 μm, coated with Xterra C18). Component characteristics were evaluated with infrared (IR) spectra using a Shimadzu Infinity FTIR spectrometer equipped with three reflectional ATR units. Nuclear magnetic resonance (NMR) experiments, including <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) were performed using an NMR spectrometer (Bruker Avance 600 MHz, BRUKER, Germany).

#### Data analysis

All data were expressed as means ± SD. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Comparison between different groups was determined using one-way or two-way analysis of variance, followed by Duncan's multiple-range test or Bonferroni's test. A *P*-value of <0.05 was considered to be significantly different. LC<sub>50</sub> (complementary log–log model) and 95% confidence intervals were obtained by probit analysis using SAS 8.01 software (Cary, NC, USA) (Kinoshita *et al.*, 1999; SAS, 2000).

## Results

### *Acaricidal activities of crude extracts from lettuce*

Polar, medium polar and non-polar compounds were extracted from different parts of lettuce (the root, leaf and seed) using different solvents (methanol, acetone and petroleum ether). As shown in table 1, extract yields of lettuce increased with solvent polarity (methanol > acetone > petroleum ether). Meanwhile, extract yields of lettuce leaves were significantly higher than those of roots and seeds.

Mite mortality significantly increased with time on treatment with extracts from different parts of lettuce. The acaricidal activity of leaf extracts was significantly higher than those of root and seed extracts. For leaf extracts, acetone extracts exhibited significantly higher acaricidal activity than petroleum ether and methanol extracts. Further, the related mite mortality induced by acetone extracts of leaves was revealed to be 63.04 and 98.89% at 48 and 72 h post-treatment, respectively (table 1).

### *Acaricidal activities of acetone extracts from lettuce leaves at different harvest months*

Since acetone extracts of lettuce leaves exhibited relatively high acaricidal activity, the effects of different harvest months were evaluated. As shown in table 2, acetone extracts of lettuce leaves harvested in July were more toxic to adult mites than those harvested in September, exhibiting LC<sub>50</sub> of 8.974, 1.429 and 0.268 mg ml<sup>-1</sup> at 24, 48 and 72 h post-treatment, respectively. In addition, acetone extracts of leaves harvested in July

exhibited higher acaricidal activities against adult mites than eggs at 48 h post-treatment (LC<sub>50</sub>: 1.429 vs. 4.829 mg ml<sup>-1</sup>). The toxicity of acetone extracts of leaves harvested in July against adult mites and eggs was almost equal to that of propargite (LC<sub>50</sub> adult: 1.429 vs. 1.083 mg ml<sup>-1</sup>; LC<sub>50</sub> egg: 4.829 vs. 3.679 mg ml<sup>-1</sup>) (table 2).

### *Acaricidal activities of different fractions of acetone extracts of lettuce leaves*

A total of 27 fractions were obtained from the acetone extracts of lettuce leaves harvested in July with column chromatography. As shown in fig. 1, mite mortalities induced by the 11th and 12th fractions (93.47 and 96.91%) were significantly higher than those induced by the other 25 fractions at 48 h post-treatment. However, yields of the 11th and 12th fractions were relatively low (1.56 and 0.71%). Besides, the 13th, 18th and 23rd fractions also exhibited relatively high acaricidal activities, and the corrected mite mortalities were 85.03, 85.06 and 84.32%, respectively. Furthermore, toxicity regression analyses showed that the 11th and 12th fractions exhibited significantly higher acaricidal activities against adult mites than did total acetone extracts of leaves at 48 h post-treatment (LC<sub>50</sub>: 0.751 and 1.258 vs. 1.429 mg ml<sup>-1</sup>) (table 3).

### *Acaricidal activities of different components in the 11th fraction*

A total of five components were isolated from the 11th fraction through column chromatography. Bioassays showed that the corrected mite mortality induced by these components increased with treatment time. At 48 h post-treatment, the highest mite mortality was induced by component 11-b, followed by component 11-a (89.00 and 69.35%, respectively). At 72 h post-treatment, the mortalities induced by these two components reached 100% (table 4). An LC<sub>50</sub> of 0.288 and 0.114 mg ml<sup>-1</sup> was measured for components 11-a and 11-b, respectively, at 48 h post-treatment (table 5).

### *Characterization of component 11-a*

As component 11-b was unstable, component 11-a was further characterized by NMR, IR and LC/MS. Component 11-a exhibited a colourless needle crystal form as deduced from ESI-MS (*m/z*, %) 414 [M-H<sup>+</sup>]. By comparing spectral data with literature data, component 11-a was identified as β-sitosterol (C<sub>29</sub>H<sub>50</sub>O).

## Discussion

Recently, the application of synthetic chemical acaricides has increased pest resistance and environmental pollution. Researchers have shifted their attention to phytochemical studies to discover efficient and environmentally compatible extracts with low toxicity and acaricidal activities (Afify *et al.*, 2011). Secondary metabolites of some medicinal plants, such as neem and chrysanthemum, with potent bioactivities against pests have been identified and exploited to protect crops in field (Chen *et al.*, 2014; Stewart *et al.*, 2016). In this study, obvious acaricidal activity was revealed from acetone extracts of lettuce leaves harvested in July. The active component from acetone extracts of leaves with high acaricidal activity was isolated and identified as β-sitosterol. Our results provide a valuable reference for the development of novel pesticides for mite control.

Table 1. Yield of extracts from different parts of lettuce by different solvents and corrected mortalities against adult female mites.

Parts	Solvents	Yield (%) <sup>1</sup>	Corrected mortality (mean ± SE) (%)		
			24 h	48 h	72 h
Root	Petroleum ether	3.06	15.18 ± 2.68 a	42.84 ± 6.35 abc	88.53 ± 1.47 b
	Acetone	3.61	4.69 ± 4.69 bcd	53.72 ± 17.24 a	96.56 ± 1.99 ab
	Methanol	4.75	3.08 ± 0.14 cd	39.94 ± 0.28 bcd	96.48 ± 2.05 ab
Leaf	Petroleum ether	5.10	1.04 ± 1.04 cd	24.93 ± 2.03 d	61.76 ± 5.12 d
	Acetone	5.99	6.27 ± 0.39 ab	63.04 ± 7.07 a	98.89 ± 1.11 a
	Methanol	6.05	1.01 ± 1.01 cd	31.09 ± 7.31 cd	93.43 ± 0.10 ab
Seed	Petroleum ether	4.09	4.69 ± 4.69 bcd	25.98 ± 4.15 d	74.73 ± 4.75 c
	Acetone	4.45	6.25 ± 1.80 ab	27.41 ± 0.00 d	91.66 ± 5.00 ab
	Methanol	6.25	0.00 ± 0.00 d	26.96 ± 3.18 d	92.93 ± 3.26 ab

<sup>1</sup>Yield (%) = (dry weight of extract/dry weight of test plant) × 100%. Different lowercase letter presented significant differences at  $P < 0.05$  by two-way ANOVA with Bonferroni's test.

Table 2. Toxicity regression analysis of acetone extracts from lettuce leaves in July and September against adult female mites and eggs.

Treatment times (h)	Harvest time	Samples	Linear regression equation	LC <sub>50</sub> <sup>1</sup> (mg ml <sup>-1</sup> )	95% confidence interval	χ <sup>2</sup>
24	July	Adult	$y = -3.188 + 3.345x$	8.974	6.100–20.438	1.496
	September	Adult	$y = -4.376 + 4.174x$	11.178	8.810–16.122	2.312
48	July	Adult	$y = -0.542 + 3.497x$	1.429	1.174–1.741	3.805
	September	Adult	$y = -0.672 + 2.868x$	1.715	1.361–2.134	2.928
72	July	Adult	$y = 2.402 + 4.197x$	0.268	0.181–0.339	0.643
	September	Adult	$y = 2.018 + 3.981x$	0.311	0.110–0.466	3.398
48	July	Adult	$y = -0.542 + 3.497x$	1.429	1.174–1.741	3.805
	July	Egg	$y = -2.344 + 3.427x$	4.829	4.205–5.620	4.309
Propargite						
48	July	Adult	$y = 1.998 - 0.069x$	1.083	0.595–1.575	6.742
48	July	Egg	$y = 1.5332 + 2.537x$	3.679	2.945–5.126	5.322

<sup>1</sup>Presented significant differences at  $P < 0.05$  by Probit analysis.

Extraction solvents are considered to be a key influential factor in the isolation of acaricidal compounds (Lapornik *et al.*, 2005). Methanolic extracts of *Andrographis echinoides* showed maximal larvicidal and acaricidal activities against *Aedes aegypti*, *Culex quinquefasciatus* and *Rhipicephalus microplus* (Mathivanan *et al.*, 2017). Compared with methanol and chloroform extracts, petroleum ether extracts of *Momordica cochinchinensis* exhibited the highest extraction rate and the most effective acaricidal activity against *T. cinnabarinus* (Huili *et al.*, 2012). In our study, extract yields of lettuce increased with increasing solvent polarity (methanol > acetone > petroleum ether), and a relatively high acaricidal activity was revealed for acetone extracts compared with those for methanol and petroleum ether extracts. These results indicate that active acaricidal components of lettuce were more likely to be stable in acetone than in methanol and petroleum ether.

As acaricidal components may be concentrated in one part of the plant, researchers attempted to evaluate acaricidal activities of different parts of the plant, such as the root, leaf, seed and fruit (Zahir *et al.*, 2009; Dantas *et al.*, 2015). For example, acetone extract of *Artemisia annua* leaf was most toxic to mites, and the corrected mortality was 100% at 48 h post-treatment (Zhang *et al.*, 2008). Acetone extract of *J. regia* leaf exhibited the highest acaricidal activity against *T. viennensis* (LC<sub>50</sub> = 90 ppm) (Wang *et al.*, 2007a). Consistent with these studies, we found that acetone extracts of lettuce leaves have the highest acaricidal activity against adult female mites. In view of the leaf as a key organ for synthesis and metabolism,

active acaricidal components of lettuce appear to be more concentrated in leaves. In addition, the harvest time of leaves could also influence the content and activity of acaricidal compounds (Liu *et al.*, 2007a; Ferraz *et al.*, 2010). Lettuce harvested in July exhibited higher total phenolic content and antioxidant capacity than those harvested in September (Liu *et al.*, 2007b). Consistent with this, we also found significantly higher extract yields and higher acaricidal activity from lettuce leaves harvested in July than those harvested in September. As July is a growing period with a long photoperiod and high temperatures, more active secondary metabolites may be accumulated in lettuce leaves in this month than in September. Moreover, obvious ovicidal activity against *T. Cinnabarinus* eggs was first confirmed from acetone extracts of lettuce leaves harvested in July in this study. The toxicity of acetone extracts of leaves harvested in July against adult mites and eggs was almost equal to the toxicity of propargite.

Through bioassay-guided fractionation of acetone extracts of lettuce leaves harvested in July, a total of 27 fractions were obtained and the 11th fraction exhibited the highest acaricidal activity. Among five components in the 11th fraction, relatively high mite mortality was induced by components 11-a and 11-b, and component 11-b was unstable. The characteristics measured by NMR, IR and LC/MS for component 11-a were consistent with β-sitosterol (Trivedi & Choudhrey, 2011; Sen *et al.*, 2013). β-sitosterol, a tetracyclic triterpene, is a main type of phytosterols widely found in various medicinal plants. β-sitosterol exhibited obvious anti-insect activity, which could

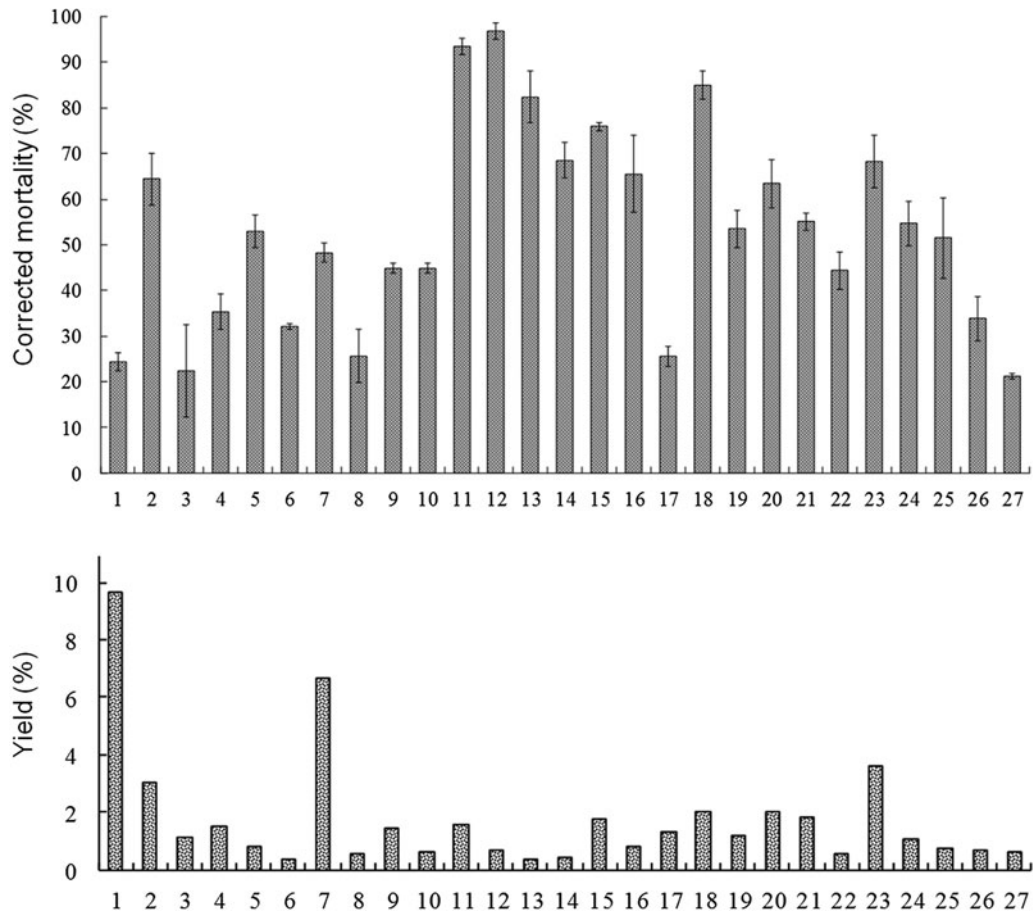


Fig 1. Yield of extracts from different fractions of acetone extracts of lettuce leaves in July and related corrected mortalities against adult female mites at 48 h post-treatment ( $1 \text{ mg ml}^{-1}$ ).

Table 3. Toxicity regression analysis of the 11th and 12th fractions from acetone extracts of lettuce leaves in July against adult female mites.

Materials	Linear regression equation	$LC_{50}^1$ ( $\text{mg ml}^{-1}$ )	95% confidence interval	$\chi^2$
Acetone extract	$y = -0.542 + 3.497x$	1.429	1.174–1.741	3.805
11th fraction	$y = -0.349 + 2.811x$	0.751	0.555–1.067	4.562
12th fraction	$y = -0.177 + 1.775x$	1.258	0.835–2.575	4.320

<sup>1</sup>Presented significant differences at  $P < 0.05$  by Probit analysis.

Table 4. Corrected mortality of female adult mites treated with different compounds of the 11th fraction at  $1 \text{ mg ml}^{-1}$ .

Treatment times (h)	Corrected mortality (mean $\pm$ SE) (%)				
	Compound 11-a	Compound 11-b	Compound 11-c	Compound 11-d	Compound 11-e
24	16.13 $\pm$ 3.23 ab	21.70 $\pm$ 5.57 a	1.61 $\pm$ 1.61 c	14.99 $\pm$ 2.87 ab	7.62 $\pm$ 0.95 bc
48	69.35 $\pm$ 1.61 b	89.00 $\pm$ 1.91 a	40.32 $\pm$ 1.61 e	52.94 $\pm$ 2.94 d	60.40 $\pm$ 1.05 c
72	100.00 $\pm$ 0.00 a	100.00 $\pm$ 0.00 a	91.49 $\pm$ 1.70 a	92.10 $\pm$ 1.69 a	89.66 $\pm$ 0.18 b

Different lowercase letter presented significant differences at  $P < 0.05$  by one-way ANOVA with Duncan' multiple test.

Table 5. Toxicity regression of compounds 11-a and 11-b from the 11th fraction against adult female mites.

Materials	Treatment time (h)	Linear regression equation	LC <sub>50</sub> <sup>1</sup> (mg ml <sup>-1</sup> )	95% confidence interval	χ <sup>2</sup>
Compound 11-a	24	$y = -0.571 + 3.132x$	1.522	1.202–2.117	1.289
	48	$y = 1.723 + 3.190x$	0.288	0.165–0.505	2.950
Compounds 11-b	24	$y = -0.238 + 2.900x$	1.208	0.960–1.613	2.570
	48	$y = 2.027 + 2.149x$	0.114	0.058–0.167	1.014

<sup>1</sup>Presented significant differences at  $P < 0.05$  by Probit analysis.

directly induce metabolic imbalance and protein changes in insects (Bu *et al.*, 2015). It has been reported that an insecticidal ingredient isolated from *Thymus mongolicus* was identified to be β-sitosterol (Feng *et al.*, 2009). Insecticidal, anti-feeding and fungicidal activities were exhibited by β-sitosterol from *Nothofagus dombeyi* and *N. pumilio* (Thoison *et al.*, 2004). β-sitosterol exhibited obvious larvicidal effects on *A. aegypti* L, *Anopheles stephensi* Liston and *C. quinquefasciatus*, and related LC<sub>50</sub> values were revealed to be 11.49, 3.58 and 26.67 ppm, respectively (Abdul *et al.*, 2008). Petroleum ether extracts of *M. piperita* were effective against *T. Cinnabarinus*, exhibiting a mortality rate of 87.05% against adult mites and 93.16% against eggs, and the principal acaricidal component was β-sitosterol (LC<sub>50</sub> = 0.546 mg ml<sup>-1</sup>) (Ren *et al.*, 2009b). All these studies are consistent with our results and further illustrate β-sitosterol as an active acaricidal component in lettuce leaves and a natural acaricidal agent to control mites.

In conclusion, acetone extracts of lettuce leaves harvested in July exhibited relatively high acaricidal activities. The active acaricidal compound of lettuce leaf was identified to be β-sitosterol. Our findings lay the foundation for the development of novel phyto-genic acaricides without hazard to humans and the environment. However, this study was performed only in the laboratory and field experiments are still needed. Meanwhile, the mechanisms of β-sitosterol's acaricidal activity still need to be studied.

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### Conflict of interest

All authors declare that they have no conflict of interest to state.

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