

# Acaricidal active fractions from acetone extract of *Aloe vera* L. against *Tetranychus cinnabarinus* and *Panonychus citri*

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**Abstract** This study aimed to isolate acaricidal active fractions from acetone extract of *Aloe vera* L. and investigate the toxicity of these fractions against *Tetranychus cinnabarinus* (*T. cinnabarinus*) and *Panonychus citri* (*P. citri*). Acetone extract of *A. vera* L. was isolated by immersing in acetone for 72 h, and diverse fractions were fractionated by column chromatography. The acaricidal activity of each fractions was evaluated by corrected mortality of *T. cinnabarinus* through slide-dip bioassay. The 8th and 13th fractions of acetone extract with good acaricidal activity were indentified by LC/MS, and the toxicity of these two fractions to *T. cinnabarinus* and *P. citri* was identified by regression analysis. Acetone extract of *A. vera* L. exhibited obvious acaricidal activity, from which a total of 18 fractions were isolated. The 8th and 13th fractions with strong acaricidal activity against *T. cinnabarinus* were identified to be 3-*O*- $\alpha$ -D-mannopyranosyl-D-mannopyranose (OAMM) and aloe emodin. When compared with spirotetrameth, both OAMM and aloe emodin exhibited higher toxicity to *T. cinnabarinus*, while only OAMM exhibited a higher toxicity to *P. citri* ( $P < 0.05$ ). OAMM and aloe emodin isolated from acetone extract of *A. vera* L. exhibited obvious acaricidal activities against *T. cinnabarinus* and *P. citri*.

**Keywords** Acetone extract · *A. vera* L. · Acaricidal activity · Mites

## Introduction

*Tetranychus cinnabarinus* (*T. cinnabarinus*) and *Panonychus citri* (*P. citri*) are most common and serious mite pests worldwide, which mainly infest crops and fruits. All the two mite pests can seriously damage the leaves of plants, resulting in substantial economic loss (Ouyang et al. 2012; Zhang et al. 2016). In general, mites in field are usually controlled by the use of various chemical acaricides (Marcic 2012; Senegačnik 2000). However, due to the short life cycle and high reproductive ability of mites, the development of drug resistance greatly limits the use of chemical acaricides (Stumpf and Nauen 2001). Meanwhile, the extensive and long-term use of chemical pesticides is also harmful to humans and the environments (Ding et al. 2013). Therefore, development of effective, safe, anti-resistant and eco-friendly alternatives for control of mite is urgently needed.

Recently, natural compounds from plant extracts have been considered to be potential alternatives of traditional chemical pesticides in control of mites (Liang et al. 2003; Schmutterer 1997). Acaricidal activity is revealed on various bioactive natural compounds, including plant essential oils (Pontes et al. 2007), plant extracts (Zhang et al. 2016) and microbial secondary metabolites (Villanueva and Walgenbach 2006). *Aloe vera* L. is a plant species of the genus *Aloe*, and mainly grows in tropical climates around the world, exhibiting obvious antifungal ability and insecticidal ability (Arunkumar and Muthuselvam 2009; Omotoso 2008). It has been reported, *A. vera* L. extract containing acaricidal and repellent bioactive components

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was useful in the control of phytophagous mites (Zhang et al. 2013); the acetone extract of *A. vera* L. showed strong acaricidal activity, causing 80.39–92.16% mortality at 72 h after treatment (Wei et al. 2011). Hexane extract of *A. vera* L. exhibited high repellency (78.75%) to *Tetranychus urticae* at 96-h post-treatment (Elkhayat et al. 2014). However, the special active ingredients of *A. vera* L. extract with high acaricidal activity were still unclear.

In this study, diverse fractions from acetone extract of *A. vera* L. were isolated, and the acaricidal activities of these fractions were evaluated. Meanwhile, two fractions with obvious acaricidal activity were identified, and their acaricidal roles in control of *T. cinnabarinus* and *P. citri* were evaluated. Our findings may not only reveal the special active ingredients of *A. vera* L. in control of mites, but also provide alternative phyto-genic acaricides without drug resistance, residual agrochemicals and environmental pollution.

## Materials and methods

### Plant material

*A. vera* L. at 4-years-old were collected from the experimental farm of Southwest University in Beibei, Chongqing, China on July 2012. A voucher specimen (No.CPP53925) has been deposited in the herbarium of the Plant Protection College, Southwest University, Chongqing, China.

### Extraction and isolation

Fresh *A. vera* L. leaves (1 kg) were dried at 40 °C, pulverized into powder using a crusher, and filtered with a 5 mm diameter mesh. Then, these samples were immersed in 100% acetone (5.0 L) (ChengDu Kelong Chemical Reagents Co., Ltd., ChengDu, China) for 72 h. After filtered with 0.5 mm diameter mesh, the acetone extract of *A. vera* L. was isolated and stored in a refrigerator at 4 °C until use. For composition analysis, acetone extract was fractionated using column chromatography on silica Gel (100–200 mesh) (Waters 2487-ZQ 4000, Qingdao Haiyang Chemical Co., Ltd., Shandong, China). Diverse fractions of acetone extract were eluted separately with different proportions of petroleum ether and ethyl acetate acid. The acaricidal activities of each fractions were evaluated, and fractions with good acaricidal activity were further analyzed.

### Substance identification

Substance identification was performed on the 8th and 13th fractions of acetone extract with good acaricidal activity.

After recrystallized in methanol (8th: white acicular crystal) and hot ethanol (13th: orange red acicular crystal), the structures of these two fractions were analyzed by LC/MS (liquid chromatography/mass spectrometry) with a fused silica capillary column (10 cm, 2.1 mm, 3.5 µm, coated with Xterra C18) on chromatograph (Waters 2487-ZQ 4000). Meanwhile, the characteristics of these two fractions were identified by Element Analyzing, UV (ultraviolet), IR (infrared), EI-MS (electron impact ion source mass spectrometry), <sup>1</sup>H and <sup>13</sup>C NMR (nuclear magnetic resonance).

### Mites

*T. cinnabarinus* (female) was originally collected from cowpea plants (*Vigna unguiculata*) in an experimental farm of Southwest University, Beibei, Chongqing, China. These mites were maintained on cowpea seedlings in an incubator at 26 ± 1 °C with 75–80% relative humidity (RH) and 14-h light/10-h dark cycle. *P. citri* (female) was originally collected from citrus nursery in Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China, which were maintained on *Citrus reticulata* seedlings in an incubator at 25 ± 1 °C with 75–80% RH and 14-h light/10-h dark cycle. All these mites were maintained under acaricide-free condition.

### Mite bioassays

A slide-dip bioassay was used to evaluate the mortality of *T. cinnabarinus* in contact with different fractions of acetone extract. The acetone extract and different fractions mixed with 0.5% acetone and 1% Tween-80 were diluted with water to 1 mg/ml as test solutions. Water containing 0.5% acetone and 1% Tween-80 was used as control. Adult mites were adhered to the dorsal surface of a glass slide. By dipping into each test solutions for 5 s, the glass slide with mites was maintaining at 26 ± 1 °C with 65–80% RH for 4 h. After 24 h and 48 h of exposure, the mites were observed and counted under a stereomicroscope (4×). The mites were considered to death when they did not respond to a gentle probe. Three replicates were made for each tested fractions.

Corrected mortality was calculated as followed:

$$\text{Corrected mortality (\%)} = \frac{\% \text{test mortality} - \% \text{control mortality}}{100 - \% \text{control mortality}}.$$

### Statistical analyses

Corrected mortality was expressed as mean ± SD, and transformed to arcsine square-root value for statistical analysis. Comparison between different groups was

determined by one-way ANOVA using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). A *P* value less than 0.05 was considered to be significantly different. The toxicity of acetone extract, OAMM (3-*O*- $\alpha$ -D-mannopyranosyl-D-mannopyranose), aloe emodin and spirodiclofen (Qingdao Hansheng Co., Ltd., Shandong, China) to *T. cinnabarinus* and *P. citri* was analyzed by regression analysis using SAS 8.01 (Cary, NC, USA) (SAS institute, 2000). The median lethal concentration (LC<sub>50</sub>) of these substances was determined at 48-h and 72-h post-treatment, respectively.

## Results

### Toxicity of acetone extract of *A. vera* L. to *T. cinnabarinus* and *P. citri*

The toxicity of acetone extract from *A. vera* L. was firstly evaluated by LC<sub>50</sub>. As a result, a good acaricidal activity was exhibited on acetone extract of *A. vera* L.. The LC<sub>50</sub> of acetone extract against *T. cinnabarinus* and *P. citri* was 0.836 and 0.613 mg/mL at 48-h post-treatment, respectively. The LC<sub>50</sub> of acetone extract against *T. cinnabarinus* and *P. citri* was 0.167 and 0.242 mg/mL at 72-h post-treatment, respectively (Tables 3, 4).

### Mortality of *T. cinnabarinus* in contact with different fractions of acetone extract of *A. vera* L.

Through column chromatography, a total of 18 fractions with different appearances were isolated from the acetone extract of *A. vera* L. (Table 1). Then the acaricidal activities of these fractions were evaluated by the mortality of *T. cinnabarinus*. As shown in Table 2, the mortality of *T. cinnabarinus* was relatively low with the treatment of different fractions for 24 h (ranged from 1.50 to 49.16%). After 48 h of treatment, the mortality of *T. cinnabarinus* reached to 47.37% at least. Importantly, the strongest acaricidal activity against *T. cinnabarinus* was exhibited on the 13th fraction (mortality: 98.38  $\pm$  1.62%). Meanwhile, the 8th, 12th and 17th fractions also exhibited relatively high acaricidal activity against *T. cinnabarinus* (mortality: 82.78  $\pm$  11.12, 84.42  $\pm$  1.74, 83.64  $\pm$  0.96) (Table 2). As the 12th and 17th fractions were not stable, only the 8th and 13th fractions were used for further researches.

### Characterization of the 8th and 13th fraction

The 8th and 13th fractions with good acaricidal activity against *T. cinnabarinus* were firstly identified by LC/MS. As shown in Fig. 1a, b, a molecular weight of 340 and 270 was revealed on the 8th and 13th fraction, respectively.

Combined with element analysis, UV, IR, EI-MS and NMR, the 8th and 13th fraction was identified to be OAMM (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) and 1, 8-Dihydroxy-3-hydroxymethyl anthraquinone aloe emodin (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>), respectively (Fig. 1c, d). The characteristics of OAMM and aloe emodin were shown as followed:

OAMM-Yield: 2.53%; appearance: yellow-green cream; m.p. 131.7–132.5 °C; IR v<sub>max</sub> (KBr) cm<sup>-1</sup>: 3423 (OH), 2932, 2 850 (Ar-H), 1133 (C–O–C), 1110, 1061 (C–O); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 Hz)  $\delta$ : C1 5.308;  $\alpha$ -type glycosidic bond; ESI-MS (*m/z*, %): 342 [M–H<sup>+</sup>, 100]; Anal. Found: C 40.05, H 6.60, N 0.22%; Calcd.: C 42.1, H 6.43, N 0%.

Aloe emodin-yield: 2.14%; Appearance: tan cream; m.p. 223–224 °C; IR v<sub>max</sub> (KBr) cm<sup>-1</sup>: 3329 (OH), 2920, 1572 (CH<sub>2</sub>), 1572, 1456 (Ar C–C), 1058 (C–O), 1675, 1627 (C = O), 1273 (Ar C = O); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 Hz)  $\delta$ : 4.62 (s, 2, *J* = 1.3 Hz, CH<sub>2</sub>), 5.60 (t, 1, *J* = 2.8 Hz, OH), 7.26 (s, 1, H-2), 7.36 (d, 1, *J* = 2.1 Hz, H-7), 7.66 (d, 1, *J* = 1.8 Hz, H-4), 7.69 (s, 1, H-5), 7.78 (t, 1, *J* = 4.0 Hz, H-6), 11.88 (s, 1, Ar–H), 11.94 (s, 1, Ar–H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 400 Hz)  $\delta$ : 62.531, 114.855, 116.306, 117.553, 119.780, 121.119, 124.826, 133.523, 133.754, 137.767, 154.174, 161.800, 162.087, 181.837, 192.066; ESI-MS (*m/z*, %): 270 [M–H<sup>+</sup>, 100]; Anal. Found: C 66.65, H 3.75, N 0.28%; Calcd.: C 66.67, H 3.7, N 0%.

### Toxicity of OAMM and aloe emodin against *T. cinnabarinus* and *P. citri*

The toxicity of OAMM and aloe emodin against *T. cinnabarinus* and *P. citri* was further analyzed by regression analysis. As a result, the LC<sub>50</sub> of OAMM and aloe emodin against *T. cinnabarinus* was 0.288 and 0.484 mg/mL at 48-h post-treatment, respectively. The LC<sub>50</sub> of OAMM and aloe emodin against *T. cinnabarinus* was 0.028 and 0.058 mg/mL at 72-h post-treatment, respectively. When compared with spirodiclofen (48 h: 0.661 mg/mL; 72 h: 0.079 mg/mL), both OAMM and aloe emodin exhibited higher toxicity to *T. cinnabarinus* (*P* < 0.05). Meanwhile, OAMM exhibited a higher toxicity to *T. cinnabarinus* than aloe emodin (Table 3).

On the other hand, the LC<sub>50</sub> of OAMM and aloe emodin against *P. citri* was 0.435 and 0.328 mg/mL at 48-h post-treatment, respectively. The LC<sub>50</sub> of OAMM and aloe emodin against *P. citri* was 0.011 and 0.017 mg/mL at 72-h post-treatment, respectively. When compared with spirodiclofen (48 h: 0.325 mg/mL; 72 h: 0.029 mg/mL), only OAMM exhibited a higher toxicity to *P. citri* (*P* < 0.05). However, no significant difference on the toxicity to *P. citri* was revealed between spirodiclofen and aloe emodin (Table 4).

**Table 1** Different fractions isolated from the acetone extract of *Aloe vera* L. leaves

Part	Merge components	Eluent proportion (v/v)	Extract appearance	Extract rate (%) <sup>a</sup>
1	(8)	10:1.0	Orange oil	7.87
2	(9)	10:1.0	Yellow oil	3.29
3	(10–13)	9.5:1.5	Orange cream	2.27
4	(14–16)	9.5:1.5	Yellow cream	2.53
5	(17–21)	9.5:1.5	Yellow–green cream	3.27
6	(22–25)	9.5:1.5	Yellow–green cream	1.51
7	(26–28)	9:2	Blackish green cream	1.40
8	(29–32)	9:2	Yellow–green cream	2.97
9	(33–38)	8.5:2.5	Yellow cream	2.83
10	(39–42)	8.5:2.5	Yellow–green cream	2.14
11	(43–49)	8.5:2.5	Yellow–green cream	2.00
12	(50–52)	8:3	Yellow–green cream	0.74
13	(53–62)	8:3	Tan cream	1.94
14	(63–74)	7.5:3.5	Tan cream	1.70
15	(75)	7:4	Puce cream	0.25
16	(76–79)	7:4	Puce cream	0.44
17	(80)	7:4	Tan cream	0.20
18	(81–82)	7:4	Tan cream	0.31

<sup>a</sup> Extract rate = (dry weight of extract/dry weight of test plant) × 100

**Table 2** Mortality of *T. cinnabarinus* in contact with different fractions (1.0 mg/mL) of acetone extract from *Aloe vera* L. leaves

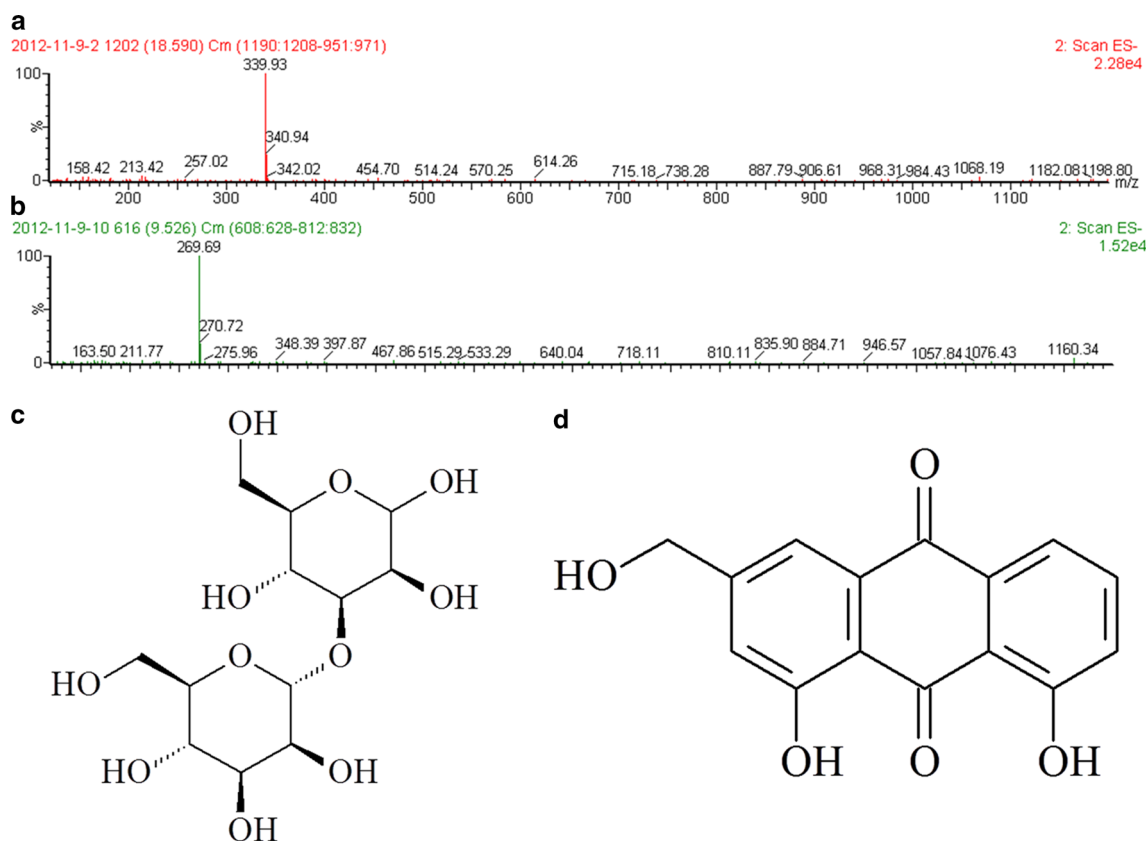
Fraction	R <sub>f</sub> value	Corrected mortality of 24 h ± SE (%)	Corrected mortality of 48 h ± SE (%)
1	0.7, 0.82	10.77 ± 3.45bc	50.75 ± 9.37abc
2	0.68	9.74 ± 5.72bc	76.98 ± 2.24abc
3	0.65	7.32 ± 2.34bc	47.37 ± 1.56abc
4	0.63	20.73 ± 6.81bc	65.26 ± 12.48bcd
5	0.6	22.68 ± 1.22bc	70.15 ± 10.37bcd
6	0.48	25.40 ± 14.92b	81.66 ± 3.06ab
7	0.54	23.81 ± 3.56b	71.43 ± 4.09bcd
8	0.5	47.19 ± 4.74bc	82.78 ± 11.12ab
9	0.48	19.4 ± 3.22bc	53.73 ± 5.67bcd
10	0.4, 0.08	11.40 ± 9.34bc	77.72 ± 2.80abc
11	0.38, 0.12	12.24 ± 8.31bc	69.63 ± 3.26abc
12	0.28, 0.08	20.53 ± 5.07bc	84.42 ± 1.74ab
13	0.28, 0.18, 0.08	49.16 ± 3.28a	98.38 ± 1.62a
14	0.27, 0.12	17.94 ± 6.71bc	51.62 ± 6.82cd
15	0.22, 0.07	6.07 ± 1.18bc	74.08 ± 9.70bcd
16	0.17, 0.05	1.50 ± 0.22c	48.44 ± 15.84d
17	0.1	14.71 ± 2.63bc	83.64 ± 0.96ab
18	0.08	10.22 ± 1.86bc	57.49 ± 4.41bcd

Values in the same column with different lowercase letters differ significantly at  $P < 0.05$  by Duncan's multiple-range test

## Discussion

In agricultural production, chemical pesticides have been widely used in the control of mites due to their excellent characteristics, such as wide variety, rapid action and low cost (Gay 2012). However, irrational use of chemical

pesticides have resulted in various issues, including drug resistance, environmental pollution and pesticide residues (Daizyr et al. 2009). Therefore, the development of high-efficiency, anti-resistance and low-residual pesticides was urgently needed. Recently, natural, plant-based botanical pesticides have emerged to be effective and safe in control



**Fig. 1** LC/MS (liquid chromatography/mass spectrometry) analysis of the 8th (a) and 13th (b) fractions from acetone extract of *Aloe vera* L. leaves. Chemical structures of OAMM (3-*O*- $\alpha$ -D-mannopyranosyl-D-mannopyranose) (c) and aloe emodin (d) were shown

**Table 3** Regression analyses of the toxicity of acetone extract, OAMM (3-*O*- $\alpha$ -D-mannopyranosyl-D-mannopyranose), aloe emodin and spirodiclofen against *T. cinnabarinus*

Fraction	Time (h)	Linear regression equation	LC <sub>50</sub> and 95% confidence interval (mg/mL)	$\chi^2$	df	P
Acetone extract	48	$y = 0.169 + 2.114x$	0.836 (0.716–0.964)	7.992	5	0.046
	72	$y = 1.605 + 2.062x$	0.167 (0.094–0.233)	3.177	5	0.365
OAMM	48	$y = 3.145 + 0.754x$	0.288 (0.126–0.659)	1.690	5	0.158
	72	$y = 2.565 + 1.770x$	0.028 (0.011–0.049)	2.560	5	0.132
Aloe emodin	48	$y = 0.454 + 1.439x$	0.484 (0.505–2.542)	35.144	5	0.000
	72	$y = 1.514 + 1.191x$	0.058 (0.024–0.153)	10.813	5	0.013
Spiroclifofen	48	$y = 0.296 + 1.881x$	0.661 (0.546–0.785)	16.426	5	0.001
	72	$y = 1.973 + 1.594x$	0.079 (0.617–1.018)	13.223	5	0.024

LC<sub>50</sub> is the lethal concentration that killed 50% of *T. cinnabarinus*. Each extract solution had five concentrations, and tests were replicated three times

of mites (Bakkali et al. 2008; Isman 2006). In this study, a good acaricidal activity was exhibited on acetone extract of *A. vera* L., which presented the acaricidal role of *A. vera* L. in mites as previous descriptions (Wei et al. 2011).

In evaluation of active ingredients in *A. vera* L., a previous study showed that the 10th and 11th fractions of *A. vera* L. extract exhibited strong acaricidal activity, with LC<sub>50</sub> values of 44 ppm and 33 ppm, respectively (Marcic 2012). However, special components of these acaricidal fractions were still unclear. In this study, a total of 18

fractions were isolated from the acetone extract of *A. vera* L. through column chromatography. Importantly, obvious acaricidal activity against *T. cinnabarinus* was exhibited on the 8th and 13th fractions. Through LC/MS, these two fractions were finally identified to be OAMM and aloe emodin.

OAMM is known as a linear polymer of mannose. Although the insecticidal role of OAMM has not been reported, mannose-binding lectin is revealed to be important in plant defenses. It has been reported, a new mannose



**Table 4** Regression analyses of the toxicity of acetone extract, OAMM (3-*O*- $\alpha$ -D-mannopyranosyl-D-mannopyranose), aloe emodin and spirodiclofen against *P. citri*

Fraction	Time (h)	Linear regression equation	LC <sub>50</sub> and 95% confidence interval (mg/mL)	$\chi^2$	df	P
Acetone extract	48	$y = 5.329 + 1.549x$	0.613 (0.473–0.795)	4.236	5	0.018
	72	$y = 5.965 + 1.571x$	0.242 (0.035–0.184)	3.177	5	0.025
OAMM	48	$y = -1.487 + 0.564x$	0.435 (0.252–1.040)	1.200	5	0.109
	72	$y = -0.900 + 0.873x$	0.011 (0.0003–0.020)	2.500	5	0.150
Aloe emodin	48	$y = 0.378 + 0.781x$	0.328 (0.091–0.584)	4.515	5	0.211
	72	$y = 1.660 + 0.932x$	0.017 (0.0004–0.090)	1.539	5	0.673
Spirodiclofen	48	$y = 0.360 + 1.847x$	0.325 (0.262–0.403)	5.465	5	0.015
	72	$y = 2.176 + 1.922x$	0.029 (0.023–0.036)	4.532	5	0.026

LC<sub>50</sub> is the lethal concentration that killed 50% of *P. citri*. Each extract solution had five concentrations, and tests were replicated three times

specific agglutinin isolated from tubers of *Amorphophallus paeoniifolius* exhibited obvious insecticidal efficacy against a wide range of hemipteran insects (Gupta et al. 2012); mannose-binding lectin from yam exhibited obvious insecticidal property against *Helicoverpa armigera* (Ohizumi et al. 2009); mannose-binding lectin was a kind of pesticides resistant to proteolytic activity in insect gut (Fitches et al. 2012). Therefore, we suspected that OAMM may exhibit a similar action mechanism with mannose, thereby contributing to mites control. On the other hand, aloe emodin is an anthraquinone present in aloe latex. As known, anthraquinone derivatives exhibited various biological activities, such as antimicrobial, antifungal, purgative, anti-inflammatory, analgesic, antitumor, and hypoglycemic ability (Ravindran et al. 2012). Except for fungicidal activity, aloe emodin also exhibited obvious insecticidal activity (Boulogne et al. 2012). It has been reported, aloe emodin was one of the active anthraquinones in *Cassia alata*, which contributed to acaricidal ability (Rahman et al. Rahman et al. 2009); By the treatment of Aloe emodin, a mortality of approximately 85% on *Anopheles gambiae* larvae and *Bemisia tabaci* were revealed (Georges et al. 2008). Therefore, we suspect that the acaricidal activity of *A. vera* L. may also be attributed to aloe emodin.

In this study, the acaricidal activities of OAMM and aloe emodin were further identified on *T. cinnabarinus* and *P. citri*. Both OAMM and aloe emodin exhibited a higher toxicity to *T. cinnabarinus* when compared with spirodiclofen, while only OAMM exhibited a higher toxicity to *P. citri* than spirodiclofen. These phenomena indicated that OAMM and aloe emodin were effectively in control of *T. cinnabarinus* and *P. citri*, which could be used as an alternative to traditional spirodiclofen. Meanwhile, the acaricidal activities of OAMM and aloe emodin were considered to be diverse in different mites populations. Further researches on the application of OAMM and aloe emodin in different insect populations were still needed.

Besides, as the yield and acaricidal activity were higher in OAMM than aloe emodin, OAMM may be more suitable for mass production with wide application prospects.

In conclusion, obvious acaricidal activity was exhibited on acetone extract of *A. vera* L.. OAMM and aloe emodin were revealed to be the special active ingredients of *A. vera* L. against *T. cinnabarinus* and *P. citri*, which may be used for the development of new phytogetic acaricides.

**Author contribution statement** JL performed the statistical analysis. YZ carried out the study, together with QZ, and collected important background information. WD conceived of this study, and participated in the design and helped to draft the manuscript. All authors read and approved the final manuscript.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interests.

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