Analysis of the acaricidal activity of Acorus gramineus extract on Dermatophagoides farinae

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Research Article

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Abstract

Background Mites and their excretion are considered allergens that cause allergic rhinitis, mite dermatitis, allergic asthma, and other hypersensitivity diseases in humans and animals. To study the acaricidal activity of *Acorus gramineus* extract on *Dermatophagoides farinae*, and to isolate and identify the active components.

Results *Acorus gramineus* extract was effective in killing *Dermatophagoides*mites. The 24-hour fatality rate was 96.4% when the lowest effective concentration was 0.0625%. Six active components, A-F, were isolated and purified by silica gel column chromatography. Results showed that components D and E, identified as isoeugenol methyl ether and β-asarone, respectively, had good acaricidal effects.

Conclusion This study showed, for the first time, that isoeugenol methyl ether has significant acaricidal activity against *Dermatophagoides farinae*.

1 Introduction

Dermatophagoides farinae is a tiny Arachnid arthropod. It is widely distributed globally and can breed in large quantities in dust found in storage and living environments (A Moya R et al. 2021; Zhu et al. 2007). Mites and their excretion are considered allergens that cause allergic rhinitis, mite dermatitis, allergic asthma, and other hypersensitivity diseases in humans and animals (Agacheet et al. 2019). Although chemical acaricides are effective in killing mites, the resulting drug residues pollute people's living environments and have toxic effects on the human body (Lozano et al. 2019). In China, acaricidal research focuses on testing a variety of Chinese herbal medicines, to identify natural acaricidal active ingredients that are efficient, economical, and non-toxic to humans.

Acorus gramineus is a perennial herb belonging to the Araceae. It is widely distributed in China, where its rhizomes are used to treat epilepsy, flatulence, inflammation, and more (Mei et al. 2022; Qiu et al. 2022; Lim et al. 2012; Kim et al. 2022; Huang et al. 2013). Previous studies have shown that volatile oil components of *A. gramineus* have different strengths against many storage pests, vegetable pests, and agricultural mites (Huang et al. 2007; Yao et al. 2014), while acaricidal activity on *D. farinae* has not been reported. In this study, the oil components of *A. gramineus* were extracted to test for acaricidal activity against *D. farinae* to provide scientific evidence for the biomimetic synthesis of new acaricides and the development and utilization of plant active compounds.

2 Materials And Methods

2.1 Materials

Dried rhizomes of *A. gramineus* were purchased from a Chinese medicinal materials factory in Yulin, Guangxi. Methanol, petroleum ether, and Tween-80 were purchased from Shanghai Bioengineering Co.,

Ltd. *Dermatophagoides farinae* mites were collected and identified from flour, and subsequently purified and reared in an artificial incubator at 25 ± 1°C and RH 75%±10%.

2.2 Methods

2.2.1 Extraction of essential oil from A. gramineus

The dried rhizomes were ground into a powder using a Fwl35/177 herbal mill and 200g was placed into a glass jar. Thereafter 800ml of methanol was added and the sample was stored at 25°C for 7 days in the dark for immersion. The supernatant was filtered out with a rotary evaporator at 45°C until no solvent dripped out of the condensing tube.

After condensation, 20g of the cold-soaked methanol extract was dissolved in a 45°C water bath, 250ml of water was added followed by 250ml of petroleum ether for extraction. The sample was kept shaking for 10min and then allowed to stand and settle. After 12h, the petroleum ether was separated, filtered, and concentrated with a rotary evaporator at 45°C to obtain the essential oil components. The yield of petroleum ether extract after concentration was 20.3%.

2.2.2 Acaricidal activity

The extracted essential oil and Tween-80 were mixed in a ratio of 1:1, diluted with distilled water to 1% essential oil concentration, and then diluted into five concentrations of 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125%. Tween-80, diluted with distilled water to the same concentrations, was used as a control group.

We used Petri dishes (60mm×30mm), with filter paper at the bottom as experimental stages. Vaseline was applied to the junction between the filter paper and the inner wall of the petri dish to prevent mites from escaping. One hundred adult mites were selected and placed in the center of the filter paper of each petri dish, and 200 μL of each essential oil concentration, or control were evenly added to the filter paper. Petri dishes were placed in the incubator at 25°C and 75% relative humidity. Each experimental and control concentration was repeated three times. After 24 hours, the number of dead mites was counted and recorded. We used a needle to touch the feet of the mites, and mites that did not respond were considered dead. The corrected mortality rate was calculated as follows: Corrected mortality rate (%) = [(Death rate of treatment group - Death rate of control group)/(1 - Death rate of control group)] × 100. Data were processed with SPSS19.0 using Duncan's Multiple-Range test.

2.2.3 Separation and purification of essential oil

The essential oil of *A. gramineus* was analyzed by normal phase column pressure, and each component was collected. The high-performance liquid chromatography (HPLC) instrument configuration was as follows: System controller: Flash-A detection system; Pump: Flash-A infusion system; Detector: Flash-A detection system. Operating conditions: Mobile phase was ethyl acetate and petroleum ether solution with 10% ethyl acetate, the detection wavelength was 254nm, a normal phase silica gel column was used, and the column temperature was room temperature.

We prepared 7%, 10%, and 15% ethyl acetate of the petroleum ether solution. Different concentrations of ethyl acetate of petroleum ether were used as the mobile phase, and samples diluted 10× and 100× were measured by TLC spotting and observed at 254 nm, 302 nm, and 365 nm after 2 hours. At 254nm, four independent sample points could be observed, and the area of the last sample point was significantly larger than the rest. Therefore, the sample was diluted again to 1000×, and the sample point was still clear and well-shaped and the solution was considered a pure substance. Using a 10% concentration for elution, five different components, labeled A, B, C, D, and E, respectively were collected. Pure ethyl acetate was used to elute again, and component F was collected. The eluent was combined and spun dry to obtain a total of six components, labeled A-F. The acaricidal activity of the six separated fractions was determined as in section 1.2.2

2.2.5 Identification of the effective acaricidal components

Components D and E were identified by liquid chromatography/mass spectrometry (LC/MS) using an HPLC (Agilent Poroshell 120 EC-C18) column (4.6*100mm, 2.7μm), and nuclear magnetic resonance (NMR). This was performed using a liquid-mass combination instrument (Thermo Vanquish-Q Exactive, No. TI-0005, USA) and a superconducting pulsed Fourier Transform NMR spectrometer (Agilent 400 MR, USA). The structure of the active monomer compounds was determined by searching the standard computer spectrogram database and comparing results with the literature.

3 Results

3.1 Acaricidal activity of *A. gramineus* essential oil

The essential oil of *A. gramineus* had good acaricidal activity against dust mites, with a fatality rate of 100% at a concentration of 0.125%. The lowest effective concentration was 0.0625%, with a fatality rate of 94.33%. The fatality rate increased with an increase in the concentration of the oil (Table 1). The mortality of the control group was less than 5%.

Table 1
Efficacy of *A. gramineus* essential oil against *D. farinae* mites (mean value ± standard deviation)

Concentration (%)	24h adjusted mortality (%)
1	100.00 ± 0.00a
0.5	100.00 ± 0.00a
0.25	100.00 ± 0.00a
0.125	100.00 ± 0.00a
0.0625	94.33 ± 1.53b
0.03125	34.33 ± 2.52c

Note: Data in the same column marked with different lowercase letters indicate significant differences in acaricidal activity at different concentrations at the same time ($P < 0.05$).

3.2 Acaricidal activity components A-F

Components D and E had good acaricidal activity against *D. farinae*. The lowest effective concentration of component D was 0.125%, and the 24h corrected mortality rate was 93.67%. The lowest effective concentration of component E was 0.03125%, and the 24h corrected mortality rate was 82.67%. (Table 2). The mortality of the control group was less than 5%.

Table 2
Acaricidal activity of separated and purified products of *A. gramineus* essential oil against *D. farinae* (mean value ± standard deviation)

Component number	Concentration (%)	24h corrected mortality rate (%)
A	1	22.67 ± 1.53d
B	1	30.33 ± 2.08d
C	1	44.33 ± 1.53c
D	1	100.00 ± 0.00a
	0.5	100.00 ± 0.00a
	0.25	100.00 ± 0.00a
	0.125	93.67 ± 1.53a
	0.0625	45.67 ± 2.08c
	0.03125	23.33 ± 2.52
E	1	100.00 ± 0.00a
	0.5	100.00 ± 0.00a
	0.25	100.00 ± 0.00a
	0.125	100.00 ± 0.00a
	0.0625	100.00 ± 0.00a
	0.03125	82.67 ± 1.53b
	0.01563	40.67 ± 2.08c
F	1	3.00 ± 1.00d

Note: Data in the same column marked with different lowercase letters indicate significant differences in acaricidal activities at different concentrations at the same time ($P < 0.05$).

3.3 Identification of the effective acaricidal components

3.3.1 Physicochemical properties of components D and E

Component D is a colorless to yellowish liquid, soluble in ethanol, acetone, and most nonvolatile oils, almost insoluble in mineral oils, and insoluble in glycerol and propylene glycol. The melting point is 5.5℃ ~ 6.5℃ and the boiling point is 262℃-264℃. Component E is a colorless liquid, soluble in petroleum ether and chloroform, with a boiling point of 296℃. It is combustible, and combustion can produce irritating smoke.

3.3.2 Mass spectrometry and NMR analysis of components D and E

Substance D was consistent with the spectral data of isoeugenol methyl ether, while substance E was consistent with the spectral data of β-asarone, with a chemical formula of $C_{12}H_{16}O_3$ (Fig. 1, 2).

4 Discussion

A. gramineus is widely distributed throughout China, and its harvested plant material is sold at a low price, making it an accessible and affordable natural treatment for mites. Extracting the essential oil is also a simple process and the essential oil has significant acaricidal activity, with the lowest effective concentration reaching 0.0625%. Isoeugenol methyl ether and β-asarone, with their lowest effective concentrations at 0.125% and 0.03125%, respectively, were the main acaricidal substances we identified from the extracted essential oil. Unfortunately, β-asarone is toxic and teratogenic, may cause liver cancer, and is thus not suitable for use as an acaricide (Zhang et al. 2019; Uebel et al. 2021; Chellian et al. 2017). The European Commission has announced that β-asarone has definite toxicity, and it is not recommended to be used as a therapeutic drug alone in clinical practice (SCF et al. 2002; JECFA et al. 2008). However, isoeugenol methyl ether, also known as methoxy isoeugenol, can be used in the production of fragrances, configuration of essences, is non-toxic to the human body, and is widely used in rose, lilac, ylang-ylang, and other floral fragrances as a fixative (Lin et al. 2007). China's GB2760-1996 "Hygienic Standard for the Use of Food Additives" stipulates that it is an edible spice, mainly used for the preparation of vanilla, berry flavors, mixed spices, and also tobacco flavors. Paula and collaborators (Paula et al. 2012) reported the presence of a phenyl-propanoid derivative (E)-methyl isoeugenol and its predominance in the essential oils from *Pimenta pseudocaryophyllus*. It has been shown that isoeugenol methyl ether has anti-anxiety and anti-depression effects, and has antihypertensive and vasodilatory effects through action on cellular calcium channels (Fajemiroye et al. 2014). In addition, isoeugenol methyl ether has an attractant effect on some pests and plays an important role in pest monitoring and management (Royer et al. 2018).

Isoeugenol methyl ether is miscible with water in any proportion after adding the appropriate amount of dimethyl sulfoxide or Tween. Thus, an acaricidal solution prepared with isoeugenol methyl ether can counter the inability of the essential oil from *A. gramineus* to dilute in water directly. Isoeugenol methyl ether can also be green synthesized from eugenol as an active ingredient (Yun et al. 2011; Sun et al. 2013; Yahyaa et al. 2019), making it easier to quantify. It can be used in a spray to kill mites directly, and can also be added to bath products, washing products, and skin care products. Future studies will study the effects of isoeugenol methyl ether on the ultrastructure and enzyme activities of *D. farinae*, as well as its acaricidal mechanism on transcriptomics and proteomics. This will lay a solid foundation for the industrialization and commercialization of Isoeugenol methyl ether as an acaricide.

Declarations

Conflict of interest

The authors declare no competing financial interest.

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Figures

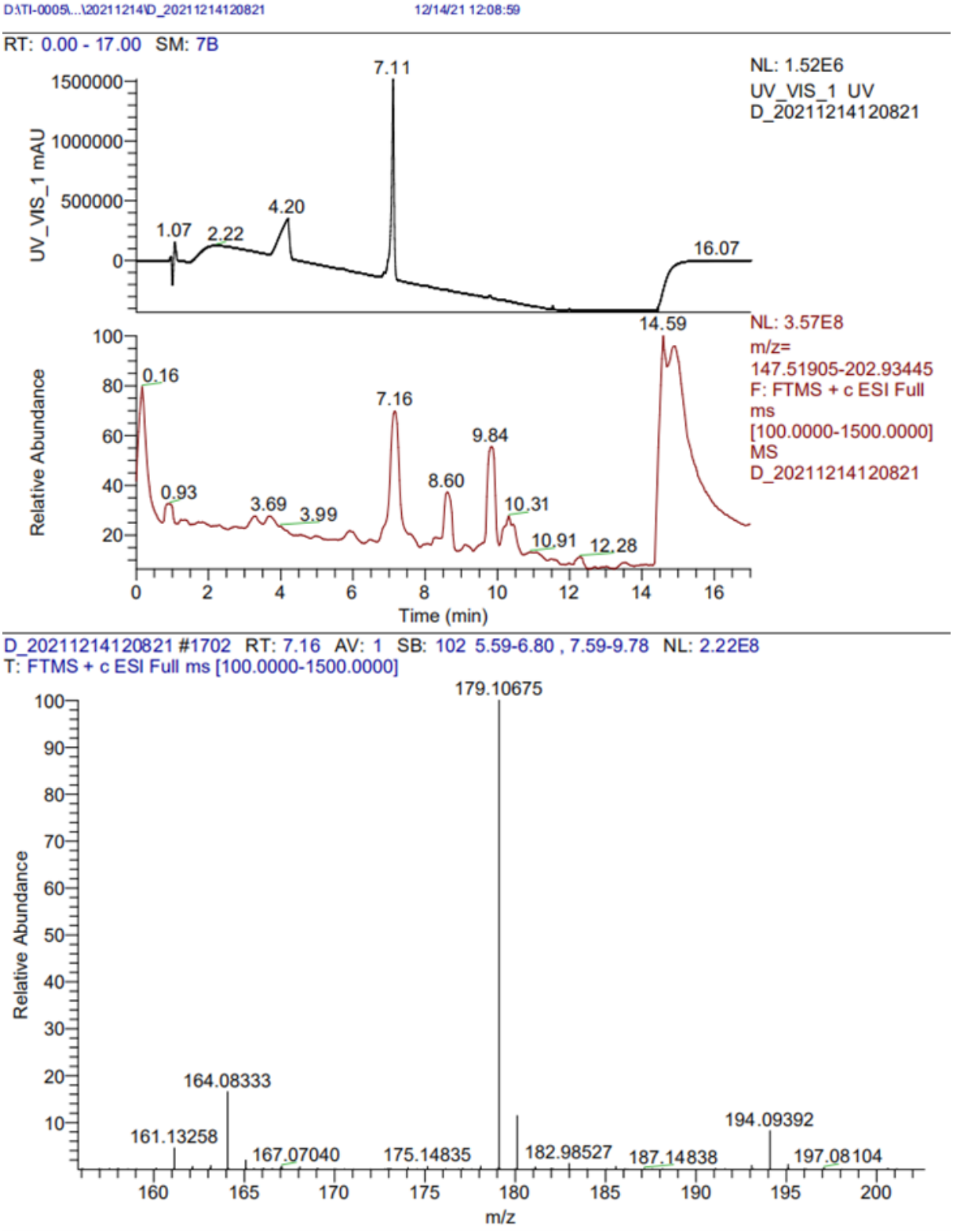

Figure 1

Mass spectrogram of component D identified from *A. gramineus* essential oil

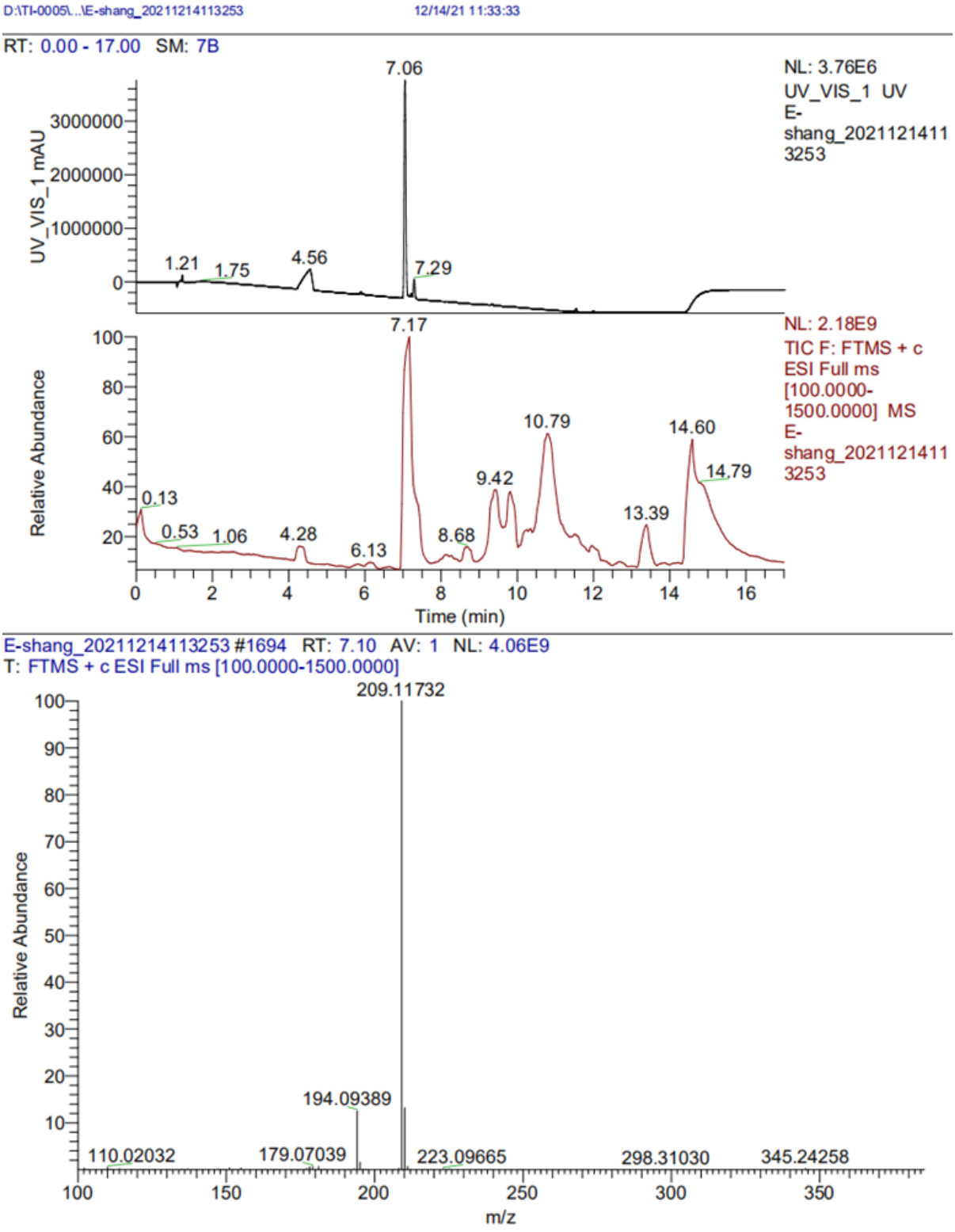

Figure 2

Mass spectrogram of component E identified from *A. gramineus* essential oil

Supplementary Files

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- 13CNMRspectraofcomponentD.png
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