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RESEARCH ARTICLE - ANTS

Insecticidal Activity of the Whole Grass Extract of *Typha angustifolia* and its Active Component against *Solenopsis invicta*

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Abstract

In this study, the toxicity of whole grass *Typha angustifolia* L. extract was determined in vitro by a "water tube" method to investigate the bioactivity of *T. angustifolia* L. against micrergates of red imported fire ants. Results indicated that the ethanol extract exhibited toxicity against the micrergates of red imported fire ants. Mortality was 100% after the micrergates were treated with 2000 mg/mL of ethanol extract for 72 h. After 48 h of treatment, LC_{50} values of ethanol extract and petroleum ether fraction were 956.85 and 398.73 mg/mL, respectively. After 120 h, LC_{50} values of the same substances were 271.23 and 152.86 mg/mL, respectively. A bioactivity-guided fractionation and chemical investigation of petroleum ether fraction yielded an active component (compound 1). NMR spectra revealed that the structure of compound 1 corresponded to 3β-hydroxy-25-methylenecycloartan-24-ol. Compound 1 also exhibited strong toxicity against the micrergates of red imported fire ants, thereby eradicating all of the tested ants treated with 240 mg/mL for 120 h. LC_{50} values of compound 1 at 48 and 120 h were 316.50 and 28.52 mg/mL, respectively.

Introduction

Typha angustifolia L. is a perennial marsh Typhaceae plant considered as a rare medicinal herb with economical and environmental importance. This plant is used as food and ornament for landscape use. *T. angustifolia* L. is a wild plant with rich natural resources cultivated and widely distributed in China. Dried pollens of *T. angustifolia* L. are commonly used as a Chinese medicinal herb (Li et al., 2011; Liu & Zhang, 2009; Yan & Xu, 1996) because these pollens have hemostatic and diuretic effects; *T. angustifolia* L. pollens can also remove blood stasis. Whole grass of *T. angustifolia* L. is also used as good medicine and food (Yu et al., 2007). Furthermore, *T. angustifolia* L. is widely used in wastewater treatment, artificial wetland system, and purified water resource (Yuan et al., 2012). Different parts of *T. angustifolia* L. are used as food with refreshing taste and high nutritive value.

Dried grass of *T. angustifolia* L. is used as good bedding for coops or poultry cages because this plant part can repel insects that parasitize warm-blooded animals, such as hens. However, reports about the bioactivity of *T. angustifolia* L. extracts or their constituents against insects have not been published yet. Previous studies on the constituents of whole *T. angustifolia* L. grass focused on flavonoids and acidic components (Gallardo-Williams et al., 2002; Jia et al., 1986; Kong et al., 2011; Zhang et al., 2008). In the present study, extracts of whole *T. angustifolia* L. grass were isolated and purified by column chromatography to identify their chemical compositions. The toxicities of the extracts and 3β-hydroxy-25-methylenecycloartan-24-ol were determined to evaluate their effectiveness to control micrergates of red imported fire ants. *Solenopsis invicta* is an aggressive invasive species. Natural products for its environmental friendly characteristic are always regarded as important alternative for synthetic organic pesticides. Thus, this study would focus on the possibility of *T. angustifolia* L. controlling red imported fire ants.

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Material and Methods

Plant material

Fresh whole *T. angustifolia* grass without flowers was collected from Wangchang Town, Qianjiang City, Hubei Province, China in September 2009 and then dried at 40 °C.

Extraction and isolation

The whole grass dried powder of *T. angustifolia* (17.51 kg) was extracted with ethanol (175 L ×3). The combined and concentrated ethanol extract was dissolved in a small amount of ethanol. The resulting mixture was resuspended in water and gradually partitioned with petroleum ether and ethyl acetate to produce 0.45 and 0.22 kg of dried organic extracts, respectively. These different solvent fractions were subjected to bioactivity assays against the micrergates of red imported fire ants. Petroleum ether-soluble fraction elicited the highest potent activity. This petroleum ether-soluble fraction (200 g) was further fractionated in a silica gel column (200- to 300-mesh column; 1 kg; 3.8 cm × 70 cm) by using a gradient mixture of petroleum ether-acetone at increasing polarities (20:1 to 1:1 and pure acetone).

Active constituent of T. angustifolia

The active compound was isolated using various chromatographic methods because of different insecticidal activities of petroleum ether fraction. The isolated compound was subjected to structural determination by spectroscopic analyses (1H NMR and 13C NMR) and by direct comparison with an authentic reference compound.

Origin and rearing of micrergate of red imported fire ants

Solenopsis invicta colonies were collected from the suburbs of Guangzhou and maintained in the laboratory for bioassays (Lv et al., 2006; Huang et al., 2007). The collected ants were fed with a mixture of 10% honey and live insects (*Tenebrio molitor* L.). A test tube (25 mm × 200 mm) partially filled with water and plugged with cotton was used as a water source. The ants were maintained in the laboratory at 25±2 °C.

Toxicity tests

"Water tube" method (Huang et al., 2007) with slight modifications was used to determine the effectiveness of *T. angustifolia* in controlling the micrergates of red imported fire ants. The ants for the toxicity tests were transferred into a beaker with a bottom diameter of 10 cm whose vertical wall coated with Fluon emulsion after drying for 24 h to prevent ants from escaping. The water source was a test tube filled with approximately two-thirds full of water and tightly fitted with a saturated cotton which was pushed into at least 3 to 5 cm from the open end of the tube.

For the toxicity tests, all the test ants were from the same colony. A group of 30 ants of them were used for each toxicity test and each test was replicated thee times. The water source tubes containing the acetone solution, ethanol extract, petroleum ether fraction, or compound 1 were placed in the beakers for the toxicity tests. Ethanol extract and petroleum ether fraction were tested at concentrations of 2000, 1000, 500, 250, and 125 µg/mL in acetone/water (1:99) mixture. Compound 1 was tested at concentrations of 240, 120, 60, 30, and 15 µg/mL in acetone/water (1:99) mixture. Acetone/water (1:99) mixture was used as a control treatment. During the tests, mortality was recorded at an interval of 24 h for a total of 120 h, and no food was provided for the micrergates of red imported fire ants.

Statistical analyses

Percent of fire ant mortality was determined and transformed to arcsine square-root values for ANOVA. Treatment means were compared and separated using Scheffe's test at $P = 0.05$. Means ± SE of untransformed data were reported.

Results and discussion

Chemical compositions of T. angustifolia

The following properties were obtained for compound 1: m. p. 155 °C to 165 °C analyzed for C30H50O2 (M+ at *m/z* 442). 1H NMR spectrum suggested that compound 1 comprised a mixture of C-24 epimers of 3β-hydroxy-25-methylenecycloartan-24-ol (Table 1) at a ratio of 60:40. 1H NMR spectrum of the major compound showed typical resonances of a cyclopropane methylene [δ 0.33 and 0.55 (AB, q, $J = 4.2$ Hz)], six methyl groups (δ 0.81–1.72), and a terminal methylene [δ 4.93 (br, s), 4.83 (br, s) (2H-26)]. These two diastereomers could not be physically separated in the present study, but spectroscopic data (Escobedo-Martínez *et* al., 2012) have revealed the diastereomeric nature of the components of compound 1. C-24 epimers of 3β-hydroxy-25-methylenecycloartan-24-ol in *T. angustifolia* L. have not been discovered yet in similar studies.

Toxicity of ethanol extract

The toxicities of different concentrations of *T. angustifolia* L. ethanol extract against red imported fire ants were determined (Fig. 1). Fig. 1 shows that 2000 µg/mL of ethanol extract completely eradicated the micrergates of red imported fire ants treated for 72 h. The corrected mortality as concentrations and treatment time were increased. As concentration was decreased to 1000 µg/mL, the ethanol extract resulted in

92.45% corrected mortality of micrergates of red imported fire ants treated for 120 h. LC50 values of the ethanol extract treated for 48 and 120 h were 956.85 and 271.23 µg/mL, respectively (Table 2).

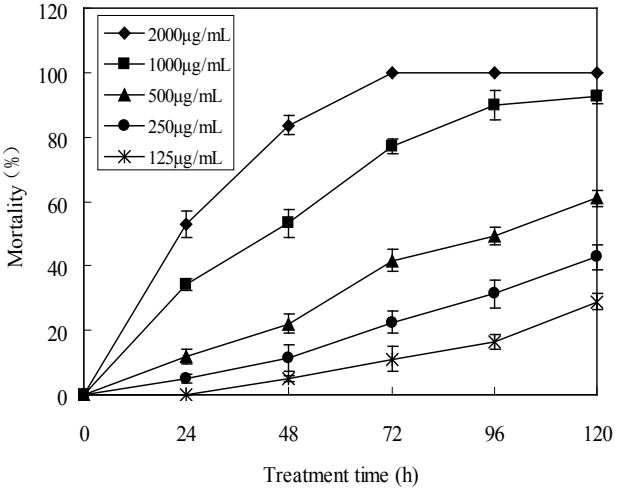

Fig. 1 Mortality of *Typha angustifolia* ethanol extract against the micrergate of red imported fire ant. Each data point represents mean ± SE of three replicates. Each replicate contains 30 tested ants.

Table 1. 1H and 13C NMR data of 3β-hydroxy-25 methylenecycloartan-24-ol.

Position	600 MHz, $CDCl_3$		150 MHz, $CDCl_3$	
	δ_H (*J*, Hz)		δ_C	
	a (major)	b (minor)	a (major)	b (minor)
1			32.11	
2			30.60	
3	3.28, m		79.06	
4			40.70	
5			47.32	
6			21.33	
7			26.23	
8			48.20	
9			20.20	
10			26.29	
11			26.68	
12			33.10	
13			45.50	
14			49.02	
15			35.77	
16	1.88, m; 1.29, m	1.88, m; 1.29, m	28.35	28.30
17			52.39	
18	0.96, s		18.25	
19	0.55, d (4.2); 0.33, d (4.2)		30.11	
20			36.13	36.16
21	0.88, d (6.0)	0.88, d (6.0)	18.54	18.53
22			32.11	
23	1.41, m; 1.61, m	1.41, m; 1.62, m	31.87	31.71
24	4.02, t (6.5)		76.58	
25			148.00	147.70
26	4.93, br, s; 4.83, br, s	4.92, br, s; 4.83, br, s	111.62	111.11
27	1.72, br, s	1.72, br, s	17.82	17.42
28	0.96, s		25.65	
29	0.81, s		14.22	
30	0.89, s		19.53	

Toxicity of petroleum ether fraction

The toxicities of petroleum ether fraction against micrergates of red imported fire ants were evaluated (Fig. 2). Petroleum ether fraction was toxic against the micrergates of red imported fire ants. In particular, 2000 µg/mL of petroleum ether fraction achieved 100% mortality against the micrergates treated for 96 h. The mortality of the micrergates treated with 500 µg/mL petroleum ether fraction for 120 h was 98.67%. LC_{50} values of petroleum ether fraction administered for 48 and 120 h were 398.73 and 152.86 µg/mL, respectively (Table 2).

Toxicity of 3β-hydroxy-25-methylenecycloartan-24-ol (compound 1) 3β-Hydroxy-25-methylenecycloartan-24-ol (compound 1) was isolated from *T. angustifolia* L. petroleum ether fraction and exhibited strong toxicity. The toxicity results of compound 1 are shown in Fig. 3. Approximately 240 µg/mL of compound 1 eradicated all of the tested ants treated for 120 h, indicating that compound 1 had strong toxicity against micrergates of red imported fire ants. Compound 1 treated for 120 h exhibited stronger toxicity at LC_{50} of 28.52 µg/mL than LC_{50} values of compound 1 treated for 48 and 120 h (Table 2). Thus, compound 1 was responsible for an excellent toxicity of *T. angustifolia* L. against micrergates of *S. invicta*.

Natural products may be considered as important alternative insecticides to control the micrergates of red imported fire ants. The results from this study demonstrated that *T. angustifolia* extracts gradually exhibited an effective toxicity. The major component in ethanol extract accounting for its toxicity was petroleum ether fraction that has an important function in controlling the micrergates of red imported fire ants. Compound 1 isolated from petroleum ether fraction also had strong toxicity. Thus, *T. angustifolia* L. ethanol extract, petroleum ether fraction, and compound 1 were effective and environmentally friendly agents that could be used to control micrergates of red imported fire ants.

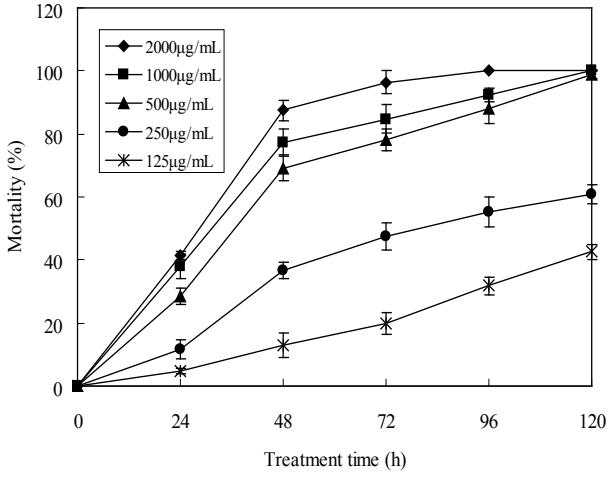

Fig. 2. Mortality of *Typha angustifolia* petroleum ether fraction against micrergates of red imported fire ants. Each data point represents mean ± SE of three replicates. Each replicate contains 30 tested ants.

Table 2. LC_{50} (μg/mL) of *Typha angustifolia* ethanol extract, petroleum ether fraction, and 3β-hydroxy-25-methylenecycloartan-24-ol against micrergates of red imported fire ants.

Treatment	48 h		120 h	
	LC_{50} (μg/mL)	95% Fiducial limit	LC_{50} (μg/mL)	95% Fiducial limit
Ethanol extract	956.85	710.43–1288.75	271.23	201.44–365.21
Petroleum ether fraction	398.73	297.60–534.23	152.86	118.41–197.34
3β-Hydroxy-25-methylenecycloartan-24-ol	316.50	146.64–683.12	28.52	20.93–38.85

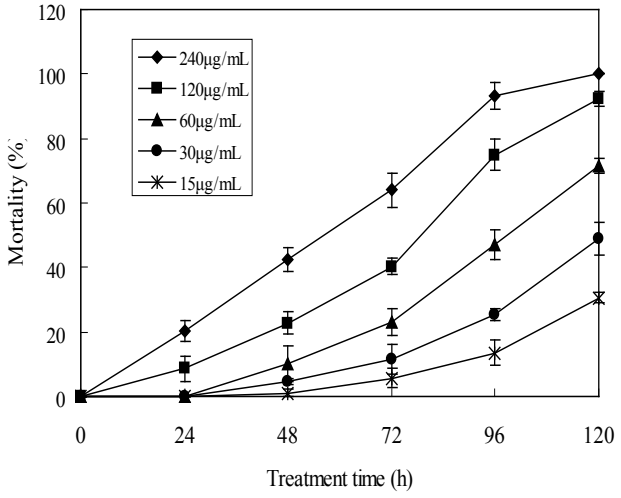

Fig. 3. Mortality of 3β-hydroxy-25-methylenecycloartan-24-ol against micrergates of red imported fire ants. Each data point represents mean ± SE of three replicates. Each replicate contains 30 tested ants.

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References

Escobedo-Martínez, C., Concepción Lozada, M., Hernández-Ortega, S., Villarreal, M. L., Gnecco, D., Enríquez, R. G., & Reynolds, W. (2012). 1H and 13C NMR characterization of new cycloartane triterpenes from *Mangifera indica*. Magn. Reson. Chem., 50: 52-57. doi: 10.1002/mrc.2836

Gallardo-Williams M. T., Geiger C. L., Pidala J. A. & Martin D. F. (2002). Essential fatty acids and phenolic acids from extracts and leachates of southern cattail (*Typha domingensis* P.). Phytochemistry, 59: 305-308. doi: 10.1016/S0031-9422(01)00449-6

Huang T. F., Xiong Z. H. & Zeng X. N. (2007). Studies on the contact toxicity of insecticides against the worker ants of *Solenopsis invicta*. J. South China Agric. Univ., 28: 26-29. (in Chinese) http://file.lw23.com/6/6a/6ab/6ab60791-9e01-4d0a-9d2c-8185732d3e22.pdf

Jia S. S., Liu Y. L., Ma C. M. , Yang S. L., Zhou H. M., Zhao D. C., Liu D. & Li S.Y. (1986). Studies on the constituents of the flavonoids from the pollen of *Typha angustfolia* L. (Puhuang). Acta Pharmac. Sin., 21: 441-446. (in Chinese) http://en.cnki.com.cn/Article_en/CJFDTOTAL-YXXB198606006.htm

Kong X. P., Chen P. D., Zhang L., Shan M. Q., Cao Y. D. & Ding A. W. (2011). Study on chemical constituents in pollen of Typha angustifolia. Jilin J. Trad. Chin. Med., 31: 72-74. (in Chinese) doi: 10.3969/j.issn.1003-5699.2011.01.038

Li J. H., Chen C. F. & Li W. W. (2011). An overview of research progress on pharmacological activity and clinical application of Pollen Typhae. J. Anhui Agric. Sci., 39: 9604-9606. (in Chinese) doi: 10.3969/j.issn.0517-6611.2011.16.056

Liu C. B., Zhang S. C. (2009). Pharmacology and clinical study on a Traditional Chinese Drug Pollen Typhae. World J. Integr. Trad. Western Med., 4: 149-152. (in Chinese). doi: 10.3969/j.issn.1673-6613.2009.02.034

Lv L. H., Feng X., Cheng H. Y., Liu J., Liu X. Y. & He Y. R. (2006). A technique for field collecting and laboratory rearing red imported fire ant, *Solenopsis invicta*. Chin. Bul. Entom., 43: 265-267. (in Chinese) doi: 10.3969/j.issn.0452-8255.2006.02.034

Yu H. F., Du L. L. & Li X. Q. (2007). Comprehensive utilization of Typha. Modern Chin. Med., 9: 31-34, 38. (in Chinese) doi: 10.3969/j.issn.1673-4890.2007.09.013

Yuan K. W., Xu W. H. (1996). Chemistry and pharmacological activity of Pollen Typhae. Chin. Trad. Herbal Drugs, 27: 693-696. (in Chinese) http://www.cnki.com.cn/Article/CJFDTotal-ZCYO199611024.htm

Yuan Y. R., Li X. Y. & Gao G. L. (2012). Research on characteristic and medical value of *Typha angustifolia* L.. Modern Agric. Sci. Technol., 9: 112-113, 115. (in Chinese) doi: 10.3969/j.issn.1007-5739.2012.09.064

Zhang S. M., Qu G. W., Xie F. X., Qiu Y. K. & Wang P. P. (2008). Study on chemical constituents in pollen of *Typha angustifolia*. Chin. Trad. Herbal Drugs, 3:, 350-352. (in Chinese) http://www.cnki.com.cn/Article/CJFDTotal-ZCYO200803009.htm