Tropical Journal of Pharmaceutical Research January 2017; 16 (1): 59-65
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i1.8

Original Research Article

Isolation of nematicidal constituents from essential oil of Kaempferia galanga L rhizome and their activity against Heterodera avenae Wollenweber

Yun Chao Li[1,2], Hua Ji[1,2], Xiu Hua Li[3], Hai Xin Zhang[4] and Hong Tao Li[1,2]*
[1]Institute of Genetics and Physiology, Hebei Academy of Agricultural and Forestry Sciences, [2]Plant Transgenic Center of Hebei Province, Shijiazhuang 050051, [3]Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, Baoding 071030, [4]Department of Gardens, Hebei Professional College of Political Science and Law, Shijiazhuang 050061, PR China

For correspondence: **Email: lihongtao1314@hotmail.com; **Tel:** +86-311-13933107622; **Fax:** +86-311-87652319*

Received: 29 July 2016 *Revised accepted: 9 December 2016*

Abstract

Purpose: *To explore the nematicidal activities of the essential oil of Kaempferia galanga rhizomes and its isolated constituents against Heterodera avenae*
Methods: *Essential oil of K. galanga rhizomes was obtained by hydrodistillation and characterized by gas chromatography/mass spectrometric (GC/MS) analysis using HP-5MS column. Evaluation of nematicidal toxicity was performed against juveniles (J2) of H. avenae. The bioactive constituent compounds were isolated and identified from the oil based on bioactivity-directed fractionation.*
Results: *Forty-one components were identified and the main components of the essential oil of K. galanga are as follows: ethyl-ρ-methoxy cinnamate (34.79 %), ethyl cinnamate (20.72%), 1,8-cineole (8.96 %), trans-cinnamaldehyde (7.03%) and borneol (5.64 %). The essential oil exhibited nematicidal activity against the cereal cyst nematode with an LC_{50} value of 91.78 µg/mL. Ethyl cinnamate, ethyl ρ-methoxy cinnamate and trans-cinnamaldehyde (median lethal concentration LC_{50} = 100.60 µg/ml, 83.04 µg/mL and 94.75 µg/mL, respectively) exhibited stronger nematicidal toxicity than borneol (LC_{50} = 734.89 µg/mL) and 1,8-cineole (LC_{50} = 921.21 µg/mL) against the cereal cyst nematode.*
Conclusion: *The results indicate that the essential oil of K. galanga and its isolated constituents have a potential for development into natural nematicides for the control of cereal cyst nematodes.*

Keywords: *Kaempferia galanga, Heterodera avenae, Nematicidal activity, Cereal cyst nematodes, Ethyl cinnamate, Ethyl ρ-methoxy cinnamate, Trans-cinnamaldehyde*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

During the current author's mass screening program for new agrochemicals from Chinese medicinal herbs, essential oil of *Kaempferia galanga* L. (Family: Zingiberaceae) rhizomes was found to possess strong nematicidal activity against cereal cyst nematodes (*Heterodera avenae* Wollenweber). Cereal cyst nematode is one of the most economically damaging endoparasite pests of wheat and causes numerous annual yield losses worldwide.

In China, the area known to be infested by the cereal cyst nematodes with potentially damaging population densities represents about 22 % of the total wheat production area. It is estimated that the overall yield suppression in the infested areas is about 10 % [1]. Control of plant nematodes has been provided principally by soil applications of conventional contact nematicides

(carbamates and organophosphates) or fumigants [2]. Increasing public concern for the environmental effects of pesticides, groundwater contamination, human health effects and undesirable effects on non-target organisms [3,4] becomes more critical on continued applications of conventional nematicides.

Essential oils and their constituents may provide an alternative to currently used nematicides/ fumigants to control plant nematodes because they are often of low mammalian toxicity, readily biodegradable and possess low danger to the environment if used in small amounts [3,4]. Many plant constituents and metabolites including essential oils have been investigated for activity against plant-parasitic nematodes [5-11].

Sand ginger (*K. galangal*) is found primarily in open areas in Southern China, Indonesia, Cambodia, Malaysia and India, but is also widely cultivated throughout Southeast Asia [12]. In China, sand ginger is well known for its popular use in the food spice and medicinal industry, traditionally treating symptoms ranging from hypertension, pectoral and abdominal pains, headache, toothache, rheumatism, dyspepsia, coughs and inflammatory tumour. It also has a long history of fragrance use to help restlessness, stress, anxiety and depression [13]. This herb has broad-spectrum biological activities including larvicidal [14], amebicidal [15], nematicidal [16-18], antimicrobial [19] and repellent effects [20]. However, to the best of our knowledge, there have been no reports to date which describe nematicidal activity of the essential oil of *K. galanga* rhizomes against the cereal cyst nematodes. Thus, the objective of this study was to investigate the chemical constituents and nematicidal activity of the essential oil against the cereal cyst nematodes and to isolate any active constituent compounds from the oil.

EXPERIMENTAL

Plant material and essential oil

The dried rhizomes of *K. galanga* (5 kg, harvested from Guangxi Zhuang Autonomous Region) were obtained from Anguo Chinese Medicinal Herbs Market (Anguo 071200, Hebei Province, China). The plant was identified, by Dr QR Liu (College of Life Sciences, Beijing Normal University, Beijing 100875, China). The voucher specimen (No. 20150901) deposited at Institute of Genetics and Physiology, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, China. The sample was ground to powder using a grinding mill (Retsch Muhle, Haan, Germany) and was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulphate was employed to eliminate water. The *n*-hexane was taken away by distillation under reduced pressure in a rotary evaporator at 35 °C and the concentrated essential oil was saved in airtight containers in a refrigerator at 4 °C for subsequent experiments.

Oil isolation and fractionation

The essential oil of *K. galanga* (25 mL) was separated on a silica gel (Merck 9385, 1,000g) column by gradient elution with a mixture of solvents (*n*-hexane, *n*-hexane-ethyl acetate, and ethyl acetate). Fractions of 500 ml were gathered and removed solvents at 40°C, and similar fractions as indicated by TLC profiles were merged to obtain 13 fractions. Fractions (3-5, 7-9) that possessed nematicidal toxicity, with alike TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until to get the pure compound for determining structure as 1,8-cineole (0.42 g), borneol (0.36 g) *trans*-cinnamaldehyde (0.27 g), ethyl cinnamate (0.44 g), and ethyl ρ-methoxy cinnamate (1.21 g).

NMR analysis

Structures of the pure compounds was determined based on nuclear magnetic resonance. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker ACF300 [300MHz (^{1}H)] and AMX500 [500MHz (^{1}H)] instruments using deuterochloroform ($CDCl_3$) as the solvent with tetramethylsilane (TMS) as the internal standard.

Gas chromatography-mass spectrometry (GC-MS)

Analyses of volatile constituents were determined using an Agilent 5973 GC-MS system operating in the EI mode at 70 eV [equipped with a 30 m HP-5MS column (0.25 mm × 30 m × 0.25 μm) and coated with 5 % phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm) (Agilent J&W Scientific, USA)]. The temperature program employed for the analysis was as follows: first temperature at 60 °C, maintained for 1 min, increased at 4 °C /min to 290 °C and remained there for 15 min. Helium was the carrier gas at 1.0 mL/min; the sample (1 μL 1/100, v/v, in acetone) was introduced in the split mode (1:10). The injector and detector temperatures were operated at 230 and 300 °C, respectively. Most constituents were recognized by gas chromatography by comparison of their

retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were estimated corresponding to a homologous series of *n*-alkanes (C_8-C_{24}) under the same operating conditions. Further identification was taken by comparison of their mass spectra with those saved in NIST 05 and Wiley 275 libraries or with mass spectra from literature [21].

Nematodes

Cysts of *H. avenae* were taken out from rhizosphere soil of wheat roots collected in Zhengzhou city (34.44 °N and 112.56 °E), Henan Province, China. Cyst masses were stocked at -4 °C for a month firstly and were maintained in Petri dishes at 15 °C during 3-7 days for the juvenile eclosion. Only freshly hatched second stage juveniles (J_2) were applyed in the experiments [22].

Nematicidal toxicity bioassay

Range-finding studies were carried on to find out the appropriate testing concentrations. A serial dilution of the essential oil and its five constituents (six concentrations, ranging from 50-1,600 µg/mL) was prepared in H_2O solution with 2 % DMSO. Aliquots of H_2O (20 µL) containing ca. 100 juveniles (J2) were moved to vials to which 980 µL of the solution containing the essential oil was put. The vials were kept in a hood at 25 °C. The nematodes were counted every 24 for 48 h. The nematodes were regarded as dead if the nematodes kept not moving and stiff after put in 1 - 2 drops of 1 % NaOH solution. Six repetitions for each treatment were carried out using H_2O and a 2 % DMSO in H_2O solution as a control. The experiments were replicated three times. Fosthiazate was purchased from National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and employed as a positive control.

Data analysis

The results from all replicates were subjected to probit analysis using PriProbit Program V1.6.3 to estimate LC_{50} values and their 95 % confidence intervals [23]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The yellow essential oil yield of *K. galanga* rhizomes was 1.18 % (v/w) and the density of the oil was determined as 0.87 g/mL. A total of 41 components of the essential oil were detected, corresponding to 98.05 % of the total oil (Table 1). The main compounds in the oil were ethyl-ρ-methoxy cinnamate (34.7 9 %), ethyl cinnamate (20.72 %), 1,8-cineole (8.96 %), *trans*-cinnamaldehyde (7.03 %) and borneol (5.64 %) followed by eucarvone (3.07 %) and δ-3-carene (3.03 %). The oil of *K. galanga* has higher content of phenylpropanoids (63.81 %) than monoterpenoids (31.18 %) and sesquiterpenoids (3.48 %) (Table 1).

1,8-Cineole (**1**, eucalyptol, Figure 1), colorless oil. ^{1}HNMR (500Hz, $CDCl_3$) δ (ppm): 1.05 (3H, 7-CH_3), 1.24 (6H, 9,10-CH_3), 1.41 (1H, H-4), 1.50 (4H, Ph-H), 1.66 (2H, Ph-H), 2.02 (2H, Ph-H). ^{13}CNMR (125Hz, $CDCl_3$) δ (ppm): 76.8 (C-8), 72.7 (C-1), 39.6 (C-4), 37.3 (C-2,6), 28.9 (C-9,10), 25.4 (C-7), 24.2 (C-3,5). The spectral data matched with the previous report [24].

Borneol (**2**,Figure 1), colorless oil.^{1}HNMR (500Hz, $CDCl_3$) δ (ppm): 1.11 (6H, 9,10-CH_3), 1.16 (3H, 7-CH_3), 1.24 (1H, H-5), 1.27 (1H, H-4), 1.42 (2H, H-2,3), 1.49 (1H, H-5), 1.52 (1H, H-4), 1.67 (1H, H-2), 3.15 (1H, H-1). ^{13}CNMR (125Hz, $CDCl_3$) δ (ppm): 76.1 (C-1), 52.7 (C-6), 50.4 (C-8), 45.23 (C-3), 35.8 (C-2), 30.0 (C-5), 23.6 (C-4), 19.8 (C-9, 10), 13.3 (C-7). The spectral data matched with the previous report [24].

trans-Cinnamaldehyde (**3**, Figure 1). Colorless oil, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 9.67 (1H, dd, *J* = 7.8 Hz, H-9), 7.53 (3H, m, H-3,4,5),7.43 (1H, m, H-7), 7.40 (2H, m, H-2,6), 6.69 (1H, m, H-8). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 145.1 (C-2), 131.9 (C-7), 124.4 (C-6), 111.7 (C-1), 73.4 (C-3), 41.6 (C-4), 27.8 (C-10), 25.7 (C-8), 22.7 (C-5), 17.7 (C-9). The data matched with previous reports [14,18].

Ethyl cinnamate (**4**, Figure 1). Colorless oil, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.30 (3H, t, *J* = 7.0 Hz, 11-CH_3), 4.22 (2H, dd, *J* = 7.2 Hz, 14.4 Hz, H-10), 6.40 (1H, d, *J* = 16.0 Hz, H-8), 7.32 (3H, m, H-3,4,5), 7.47 (2H , m, H-2, 6), 7.65 (1H, d, *J* = 16.0 Hz, H-7).^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 166.8 (C-9), 144.5 (C-7), 134.2 (C-1), 129.9 (C-4), 128.8 (C-3,5), 127.7 (C-2,6), 118.0 (C-8), 60.1 (C-10), 14.3 (C-11). The data matched those of previous reports [14,18].

Ethyl p-methoxy cinnamate (**5**, Figure 1).White needle, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.31 (3H, t, *J* = 7.0 Hz, 11-CH_3), 3.83 (3H, s, 12-CH_3), 4.23 (2H, s, H-10), 6.29 (1H, d, *J* = 16.0 Hz, H-8), 6.89 (2H, dd, *J* = 2.0 Hz, 7.0 Hz, H-2,6), 7.46 (2H, dd, *J*= 2.0 Hz, 7.0 Hz, H-3,5), 7.62 (1H, d, *J* = 16.0 Hz, H-7). ^{13}C-NMR ($CDCl_3$, 125MHz) δ (ppm): 169.2 (C-9), 163.1(C-4), 144.1 (C-7),

129.5(C-2,6),128.4 (C-1), 115.5(C-8), 114.1 (C-3,5), 60.2 (C-10), 55.1 (C-12), 14.2 (C-11). The data matched with previous reports [14,18].

The essential oil of *K. galanga* rhizomes exhibited nematicidal activity against cereal cyst nematode with an LC_{50} value of 91.78 µg/mL. Among 5 isolated constituents, three cinnamate derivates {ethyl cinnamate, ethyl ρ-methoxy cinnamate and *trans*-cinnamaldehyde (LC_{50} = 100.60, 83.04 and 94.75 µg/mL, respectively)} exhibited stronger nematicidal toxicity than two monoterpenoids (borneol, LC_{50} = 734.89 µg/mL and 1,8-cineole, LC_{50} = 921.21 µg/mL) against the cereal cyst nematode (Table 2).

Table 1: Composition of the essential oil of *Kaempferia galanga* rhizome

Peak no.	Compound	Retention index	(%)
1	α-Pinene*	931	0.48
2	Camphene	957	0.61
3	β-Pinene*	974	1.26
4	β-Myrcene*	991	0.31
5	δ-3-Carene	1008	3.03
6	α-Terpinene	1017	0.45
7	β-Cymene	1020	0.21
8	(+)-Limonene*	1028	0.60
9	1,8-Cineole*	1032	8.96
10	Acetophenone	1066	0.61
11	Linalool*	1097	0.13
12	*trans*-Pinocarveol	1138	0.82
13	Camphor*	1146	1.64
14	Borneol*	1167	5.64
15	*p*-Cymen-8-ol	1182	0.73
16	α-Terpineol*	1188	0.74
17	Verbenone	1205	0.16
18	Carvone	1238	0.71
19	Eucarvone	1250	3.07
20	*p*-Anisaldehyde	1255	0.80
21	*trans*-Cinnamaldehyde*	1266	7.03
22	Bornyl acetate	1287	0.46
23	Sabinyl acetate	1291	0.64
24	α-Copaene	1374	0.72
25	α-Gurjunene	1411	0.15
26	β-Caryophyllene*	1420	0.21
27	γ-Elemene	1433	0.18
28	(Z)-β-Farnesene	1438	0.17
29	α-Caryophyllene	1454	0.14
30	*trans*-Ethyl cinnamate	1462	1.27
31	Ethyl cinnamate*	1470	20.72
32	β-Chamigren	1478	0.19
33	γ-Muurolene	1481	0.14
34	Pentadecane	1500	0.85
35	δ-Cadinene	1523	0.29
36	Calamenene	1520	0.23
37	Spathulenol*	1578	0.31
38	Caryophyllene oxide*	1586	0.44
39	α-Cedrol	1598	0.19
40	β-Eudesmol	1648	0.12
41	Ethyl *p*-methoxy cinnamate*	1760	34.79
	Total identified		98.05
	Monoterpenoids		31.18
	Sesquiterpenoids		3.48
	Phenylpropanoids		63.81
	Others		2.26

RI, retention index as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons. *Identification by co-injection of authentic compounds

Table 2: Nematicidal activity of the essential oil derived from *Kaempferia galanga* rhizomes and its isolated constituents against *Heterodera avenae*

Treatment	LC_{50} (µg/mL)	95%fiducial limits	Slope ± SD	Chi-square (χ^2)
Essential oil	91.78	85.21-102.73	3.35 ± 0.26	5.46*
Borneol	734.89	665.45-816.56	6.31 ± 0.52	6.32*
1,8-Cineole	921.21	833.76-996.89	5.32 ± 0.43	4.58*
Ethyl cinnamate	100.60	90.63-111.76	4.32 ± 0.39	3.93*
Ethyl *p*-methoxy cinnamate	83.04	74.14-92.46	3.65 ± 0.26	7.45*
trans-Cinnamaldehyde	94.75	80.38-113.49	4.19 ± 0.36	8.07*
Fosthiazate	84.74	77.81-91.34	2.34 ± 0.21	8.54*

*Significant at $p < 0.05$ level

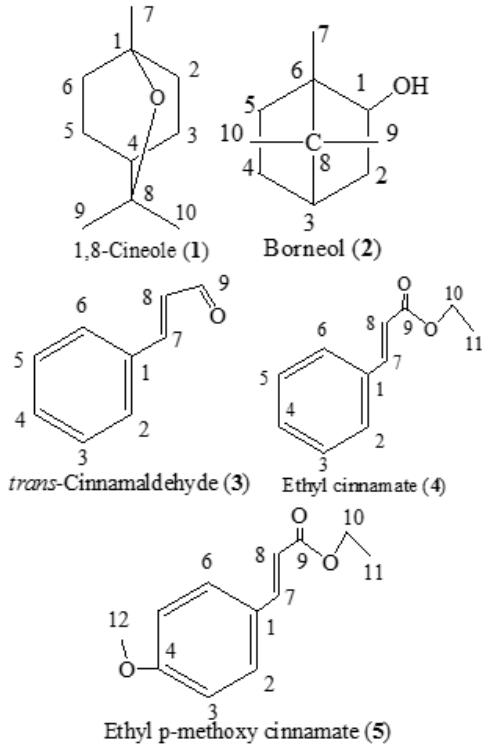

Figure 1: Constituents isolated from the essential oil of *Kaempferia galanga*

DISCUSSION

Ethyl-ρ-methoxy cinnamate, ethyl cinnamate, 1,8-cineole, *trans*-cinnamaldehyde and borneol were the major compounds in the essential oil of *K. galanga* rhizomes. Chemical composition of the essential oil of *K. galanga* in the present study was the same as that reported in previous studies [20,25,26] which ethyl ρ-methoxy cinnamate and ethyl cinnamate were always two main constituent compounds in the essential oil.

The essential oil of *K. galanga* rhizomes exhibited strong nematicidal activity against the cereal cyst nematode. Compared with the positive control, fosthiazatete (LC_{50} = 84.74 µg/mL), *K. galanga* essential oil showed the same level of toxicity (based on LC_{50} values) to the cereal cyst nematodes. Moreover, compared with the other essential oils using the same bioassay, the essential oil of *K. galanga* rhizomes possess stronger nematicidal activity against cereal cyst nematodes (*H. avenae*), such as essential oils derived from *Hyssopus cuspidatus* (LC_{50}= 338.70 µg/mL) [27], and *Valeriana amurensis* (311.6 µg/mL) [22]. This is the first report of nematicidal activities of the *K. galanga* rhizomes against cereal cyst nematodes, although the plant extract and essential oil of *K. galanga* rhizome were previously reported to display nematicidal activity against the pine wood nematode, *Bursaphelenchus xylophilus* [16] and the southern root-knot nematode, *Meloidogyne incognita* [17].

The three isolated cinnamate derivatives demonstrated almost same level of toxicity against the cereal cyst nematode as the crude essential oil (Table 2). However, the two isolated monoterpenoids, borneol and 1,8-cineole possessed weaker toxicity than the essential oil of *K. galanga* rhizomes. It is suggested that acute toxicity of the essential oil of *K. galanga* rhizomes maybe attributed to the three cinnamate derivatives (ethyl ρ-methoxy cinnamate, *trans*-cinnamaldehyde, ethyl cinnamate). Moreover, in the previous reports, the three cinnamate derivatives (ethyl ρ-methoxy cinnamate, *trans*-cinnamaldehyde, ethyl cinnamate) were revealed to exhibit nematicidal activity against the pine wood nematode, *B. xylophilus* and the southern root-knot nematode, *M. incognita* [18,28,29].

Sand ginger (*K. galangal*) is well known for its popular use in the food spice and medicinal industry [13]. It is suggested that this Chinese medicinal herb (spice) is safe for human consumption because it has been used as a spice and medicinal herb for hundreds of years. Moreover, an acute toxicity study of crude extracts of *K. galanga* and ethyl *p*-methoxy cinnamate validated that *K. galanga* extracts were safe at a dose level of 5,000 mg/kg, and

LD_{50} value was estimated to be higher than 5,000 mg/kg and ethyl *p*-methoxy cinnamate had a LD_{50} value of higher than 2,000 mg/kg [30]. The toxicity of crude rhizome extract of *K. galanga* using the Hippocratic screening test and acute and subacute toxicities in rats were also measured [31]. The results showed that extract of *K. galanga* rhizomes demonstrates less toxicity. However, no information on toxicity of the essential oil and the other constituents to human were available. Thus, to establish a practical application for the essential oil and the isolated constituents as novel nematicides, further research into the safety of the essential oil/compounds in humans is required. Additional studies on the development of formulations are also necessary to enhance the efficacy and stability and to cut down cost.

CONCLUSION

The findings of the present work suggest that the essential oil of *K. galanga* rhizomes and its isolated constituents, especially the three cinnamate derivatives, demonstrate strong nematicidal activity against the cereal cyst nematode, *H. avenae*. Thus, the essential oil and the five isolated ingredients, may find application in pest control, but further studies to determine safety in humans and to enhance its activity is required.

DECLARATIONS

Acknowledgement

The authors are grateful to Dr QR Liu, College of Life Sciences, Beijing Normal University, Beijing 100875, China, for identification of plant material used in the study. This work was funded by Ministry of Agriculture Scientific Research Special Public Welfare Industry (no. 201503114) and Talents Introduction Project of Hebei Province Finance (no. F15E 0003).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

REFERENCES

1. *Peng DL, Nicol JM, Li HM, Hou SY, Li HX, Chen SL, Ma P, Li HL, Riley IT. Current knowledge of cereal cyst nematode (Heterodera avenae) on wheat in China. In: Cereal cystnematodes: status, research and outlook. Riley IT, Nicol JM, Dababat AA (Eds), CIMMYT, Ankara, Turkey, pp 29-34, 2009.*
2. *Chitwood DJ. Phytochemical based strategies for nematode control. Annu Rev Phytopathol 2002; 40: 221-249.*
3. *Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006; 51: 45-66.*
4. *Ntalli NG, Caboni P. Botanical nematicides: A review. J Agric Food Chem 2012; 60: 9929-9940.*
5. *Bai CQ, Liu ZL, Liu QZ. Nematicidal constituents from the essential oil of Chenopodium ambrosioides aerial parts. E-J Chem 2011; 8(S1):143-148.*
6. *Du SS, Zhang HM, Bai CQ, Wang CF, Liu QZ, Liu ZL, Wang YY, Deng ZW. Nematocidal flavone-C-glycosides against the root-knot nematodes (Meloidogyne incognita) from Arisaema erubescens tubers. Molecules 2011; 16: 5079-5086*
7. *Zhang HM, Wang GL, Bai CQ, Liu P, Liu ZM, Liu QZ, Wang YY, Liu ZL, Du SS, Deng ZW. A new eudesmane sesquiterpene glucoside from Liriope muscari fibrous roots. Molecules 2011; 16: 9017-9024.*
8. *Li HQ, Bai CQ, Chu SS, Zhou L, Du SS, Liu ZL, Liu QZ. Chemical composition and toxicities of the essential oil derived from Kadsura heteroclita stems against Sitophilus zeamais and Meloidogyne incognita. J Med Plant Res 2011; 5: 4943-4948.*
9. *Liu QZ, Li HQ, Liu ZL. Nematicidal constituents from the ethanol extract of Evodia rutaecarpa Hort unripe fruits. J Chem 2013; doi: 10.1155/2013/939215.*
10. *Li HQ, Liu QZ, Liu ZL, Du SS, Deng ZW. Chemical composition and nematicidal activity of essential oil of Agastache rugosa against Meloidogyne incognita. Molecules 2013; 18:4170-4180.*
11. *Bai PH, Bai CQ, Liu QZ, Du SS, Liu ZL. Nematicidal activity of the essential oil of Rhododendron anthopogonoides aerial parts and its constituent compounds against Meloidogyne incognita. Z Naturf 2013; 68C: 307-312.*
12. *The Editorial Board of Flora China, Chinese Academy of Science. Flora of China. Science Press, Beijing, China.*

1981; vol. 16, no. 2, pp 41-42. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200028415

13. *Huang LF, Yagura T, Chen SL. Sedative activity of hexane extract of Keampferia galanga L. and its active compounds. J Ethnopharm 2008; 120:123-125.*
14. *Kim NJ, Byun SG, Cho JE, Chung K, Ahn YJ. Larvicidal activity of Kaempferia galanga rhizome phenylpropanoids towards three mosquito species. Pest Manag Sci 2008; 64: 857-862.*
15. *Chu DM, Miles H, Toney D, Ngyuen C, Marciano-Cabral F. Amebicidal activity of plant extracts from southeast Asia on Acanthamoeba spp. Parasitol Res 1998; 84: 746-752.*
16. *Choi IH, Park JY, Shin SC, Park IK. Nematicidal activity of medicinal plant extracts and two cinnamates isolated from Kaempferia galanga L. (Proh Hom) against the pine wood nematode, Bursaphelenchus xylophilus. Nematology 2006; 8: 359-365.*
17. *Hong TH, Lee JK, Heo JW, Kim SI, Choi DR, Ahn YJ. Toxicity of Kaempferia galanga rhizome-derived extract and steam distillate to Meloidogyne incognita juveniles and eggs, and their effects on Lycopersicon esculentum germination and growth. Nematology 2010; 12: 775-782.*
18. *Hong TH, Kim SI, Heo JW, Lee JK, Choi DR, Ahn YJ. Toxicity of Kaempferia galanga rhizome constituents to Meloidogyne incognita juveniles and eggs. Nematology 2011; 13: 235-244.*
19. *Norajit K, Laohakunjit N, Kerdchoechuen O. Antibacterial effect of five Zingiberaceae essential oils. Molecules 2007; 12:2047-2060.*
20. *Liu XC, Liang Y, Shi WP, Liu QZ, Zhou L, Liu ZL. Repellent and insecticidal effects of the essential oil of Kaempferia galanga rhizomes to Liposcelis bostrychophila (Psocoptera: Liposcelidae). J Econ Entomol 2014; 107: 1706-1712.*
21. *Adams RP. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured: Carol Stream, IL, USA, 2007*
22. *Li YC, Ji H, Li HT. Gas chromatography-mass spectrometric analysis of nematicidal essential oil of Valeriana amurensis P Smirn ex Kom (Valerianaceae) roots and its activity against Heterodera avenae. Trop J Pharm Res 2015, 14: 1673-1678.*
23. *Sakuma M. Probit analysis of preference data. Appl Entomol Zool 1998; 33: 339-347.*
24. *Chu SS, Du SS. Liu ZL. Fumigant compounds from the essential oil of Chinese Blumea balsamifera leaves against the maize weevil (Sitophilus zeamais). J Chem 2013; doi: 10.1155/2013/289874*
25. *Wong KC, Ong KS, Lim CL. Composition of the essential oil of rhizomes of Kaempferia galanga L. Flav Fragr J 1992; 7: 263-266.*
26. *Raina AP, Abraham Z. Chemical profiling of essential oil of Kaempferia galanga L. germplasm from India. J Essent Oil Res 2016; 28: 29-34.*
27. *Li HT, Zhao NN, Yang K, Liu ZL, Wang Q. Chemical composition and toxicities of the essential oil derived from Hyssopus cuspidatus flowering aerial parts against Sitophilus zeamais and Heterodera avenae. J Med Plant Res 2013; 7: 343-348.*
28. *Kong JO, Lee SM, Moon YS, Lee SG, Ahn AJ. Nematicidal activity of cassia and cinnamon oil compounds and related compounds toward Bursaphelenchus xylophilus (Nematoda: Parasitaphelenchidae). J Nematol 2007; 39: 31-36.*
29. *Oka Y. Nematicidal activity of essential oil components against the root-knot nematode Meloidogyne javanica. Nematology 2001; 3: 159-164.*
30. *Umar MI, Asmawi MZ, Sadikun A, Atangwho IJ, Yam MF, Altaf R, Ahmed A. Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from Kaempferia galanga L. extracts. Molecules 2012; 17: 8720-8734.*
31. *Kanjanapothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jitpakdi A, Pitasawat B. Toxicity of crude rhizome extract of Kaempferia galanga L. (Proh Hom). J Ethnopharmacol 2004; 90: 359-365.*