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Chemical constituents and insecticidal activities of the essential oil from *Alpinia blepharocalyx* rhizomes against *Lasioderma serricorne*

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Abstract: The aim of this research was to determine the chemical constituents and toxicities of the essential oil derived from *Alpinia blepharocalyx* rhizomes against the cigarette beetle, *Lasioderma serricorne* (Fabricius). The essential oil of *A. blepharocalyx* rhizomes was obtained by hydrodistillation and was investigated by gas chromatography–mass spectrometry (GC–MS). A total of 46 components of the essential oil of *A. blepharocalyx* rhizomes were identified. The principal compounds in *A. blepharocalyx* essential oil were camphor (23.13 %), sabinene (11.27 %), *α*-pinene (9.81 %) and eucalyptol (8.86 %) followed by camphene (8.05 %), sylvestrene (5.61 %) and *α*-phellandrene (5.00 %). Among them, the four active constituents, predicted with a bioactivity-test, were isolated and identified as camphor, sabinene, *α*-pinene and eucalyptol. The essential oil of *A. blepharocalyx* possessed strong contact toxicity against the cigarette beetle with ad LD_{50} value of 15.02 μg adult^{-1}, and exhibited strong fumigant toxicity against *L. serricorne* adults with an LC_{50} value of 3.83 mg L^{-1} air. The results indicate that the essential oil of *A. blepharocalyx* shows potential in terms of contact and fumigant toxicities against stored product insects.

Keywords: cigarette beetle; camphor; eucalyptol; contact toxicity; fumigant toxicity.

INTRODUCTION

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is one of the most serious pests of stored tobacco, tobacco

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products, cereal grains and processed foods throughout the world.[1] Control of *L. serricorne* populations around the world is primarily dependent upon continued applications of phosphine.[2] Although effective, its repeated use for decades has led to serious problems, including insecticide resistance, disruption of biological control by natural enemies, environmental and human health concerns, the rising cost of production and lethal effects on non-target organisms.[3,4] There is, therefore, an urgent need to find an alternative strategy for the control of these pests. Among integrated pest management tactics, plants have played a significant role because they constitute an important source of insecticides.[5] In recent years, essential oils have received much attention as pest control agents. They are characterized by low toxicity to human and animals, high volatility, and toxicity to insect pests of stored grain.[6] In a previous report,[7] it was suggested that essential oils might be applicable to the protection of stored products.

Antagonistic storage has been used as one of traditional Chinese medicinal materials conservation methods. It mainly utilizes some traditional Chinese medicinal materials having special volatile odor to store with medicinal materials vulnerable to insects, to prevent the insects. In order to inherit and develop the traditional method of prevention and control of stored grain insects, *Alpinia blepharocalyx* K. Schum, used as flavor and fragrance, a new plant resource was taken as the study sample. *A. blepharocalyx* belongs to the Zingiberaceae family and is widely distributed in the south–west of China (*e.g.*, in the Yunnan Province).[8] The rhizomes of *A. blepharocalyx* are used in Chinese traditional medicine for the treatment of abdominal distension and abdominal pain.[9] The chemical constituents of rhizomes of this medicinal herb were studied.[10] During the mass screening program for new agrochemicals from wild plants, the essential oil of *A. blepharocalyx* rhizomes was found to possess strong insecticidal activity against the cigarette beetle. A literature survey showed that there are no reports on the insecticidal activity of the essential oil of *A. blepharocalyx* rhizomes. Thus, it was decided to investigate insecticidal activities of the essential oil from *A. blepharocalyx* rhizome parts and its chemical constituents against *L. serricorne*. It was expected that this research work would provide some theoretical basis for the conception of antagonistic storage.

EXPERIMENTAL

Plant material and essential oil extraction

The fresh rhizomes (2.0 kg) of *Alpinia blepharocalyx* were harvested from Xishuangbanna (21°08'–22°36' N and 99°56'–101°50' E), Yunnan Province, China, in August 2013. The plant was identified, and a voucher specimen (BNU-dushushan-2013-08-12-24) was deposited at the Herbarium (BNU) of the College of Resources Science and Technology, Beijing Normal University, China. The sample was air-dried and ground to powders using a grinding mill. The powders were subjected to hydrodistillation using a modified clevengertype apparatus for 6 h and extracted with *n*-hexane. Anhydrous sodium sulfate was used to remove water after the extraction. The essential oil was stored in airtight containers at 4 °C for subsequent experiments.

Insects

Cultures of the cigarette beetle, *L. serricorne*, were maintained in the laboratory without exposure to any insecticide. They were reared on sterilized diet (wheat flour/yeast mass ratio of 10:1) at 29–30 °C, 70–80 % r.h. in the dark. The unsexed adult beetles used in all the experiments were about 1–2 weeks old. All containers housing insects used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon).

GC–MS analysis

The essential oil was subjected to GC–MS analysis on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and an HP-5MS (30 m×0.25 mm×0.25 μm) capillary column. The column temperature was programmed at 50 °C for 2 min, then increased at 2 °C min^{-1} to a temperature of 150 °C and held for 2 min, and then increased at 10 °C min^{-1} until the final temperature of 250 °C was reached, which was held for 5 min. The injector temperature was maintained at 250 °C. The samples (1 μL) were diluted to 1 % with *n*-hexane. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. The spectra were scanned from *m*/*z* 50 to 550. Most constituents were identified by gas chromatography by comparison of their retention indices with those in the literature or with those of available authentic compounds. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) obtained under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in the NIST 05 and Wiley 275 libraries or with mass spectra from the literature.[11] The relative percentages of the component were calculated based on the normalization method without the use of correction factors.

Isolation and characterization of the four main constituent compounds

The crude essential oil (9 mL) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (30 mm i.d., 500 mm length) by gradient elution with *n*-hexane first, then with *n*-hexane–ethyl acetate (in volume ratios of 100:1, 50:1, 20:1 and 5:1, with gradient elution), and finally with ethyl acetate to obtain 25 fractions, with flow rate of 0.50 cm^3/s; the size of each fraction was 80 g and then the solvent was changed. Based on contact toxicity, fractions 3, 9 and 15 were chosen for further fractionation. With PTLC, four purified compounds were obtained. The isolated compounds were elucidated from their NMR spectra. The NMR experiments were performed on a Bruker Avance DRX 500 instrument using $CDCl_3$ as solvent with TMS as internal standard.

Contact toxicity bioassay

The insecticidal activities of *A. blepharocalyx* essential oil and the four main compositions were determined by direct contact application. Range-finding studies were run to determine the appropriate testing concentrations. A serial dilutions (five concentrations: 2.20–10.00 % for the oil, 1.97–10.00 % for camphor, eucalyptol and sabinene and 5.93–30.00 % for α-pinene) were prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Five replicates were performed for all treatments and controls, and the experiment was replicated three times. Both treated and control insects were then transferred to glass vials (10 insects per vial) with culture media and kept in incubators. Mortality was observed after 24 h. The LD_{50} values were calculated using Probit analysis.[12]

Fumigant toxicity bioassay

Serial dilutions of the essential oil and the four main compositions (five concentrations: 0.44–2.00 % for oil, 0.59–3.00 % for camphor, 0.99–5.00 % for eucalyptol, 5.21–20.00 % for sabinene and 3.95–20.00 % for α-pinene) were prepared in *n*-hexane. A Whatman filter paper (diameter 2.0 cm) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 25 mL). Ten microliters of an appropriate concentration was added to the filter paper. The solvent was allowed to evaporate for 20 s before the caps were placed tightly on the glass vials, each of which contained 10 insects, to form a sealed chamber. Preliminary experiments demonstrated that 20 s was sufficient for the evaporation of solvents. Fluon was used inside each glass vial to prevent insects reaching the treated filter paper. *n*-Hexane was used as a control. Five replicates were performed for all treatments and controls, and they were incubated for 24 h. The experiments were repeated three times. The mortality was recorded. The LC_{50} values were calculated by using Probit analysis.[12]

RESULTS AND DISCUSSION

Chemical constituents of essential oil

The brownish red essential oil yield of *Alpinia blepharocalyx* rhizome parts was 0.45 % (*V*/*w*) and the density of the concentrated essential oil was determined to be 0.82 g mL^{-1}. GC–MS analysis of the essential oil of *A. blepharocalyx* rhizome parts led to the identification and quantification of a total of 46 major components, accounting for 96.38 % of the total components present (Table S-I of the Supplementary material to this paper). The main constituents of *A. blepharocalyx* rhizomes essential oil were camphor (23.13 %), sabinene (11.27 %), α-pinene (9.81 %) and eucalyptol (8.86 %).

However, the principal components of the essential oil from *A. blepharocalyx* rhizomes analyzed in this work differed from those in previous reports. For example, in several previous studies, cinnamic acid methyl ester was isolated and identified as the main component; it made up 90.88 % of the essential oil from *A. blepharocalyx*.[10–13] However, in another study, γ-cadinene (18.70 %), linalool (5.45 %), geranyl acetate (3.86 %) and δ-cadinene (3.08 %) were the dominant components in the essential oil of *A. blepharocalyx*.[14] These differences were possibly due to the differences in the place of origin and plant parts used.

Structure confirmation of isolated compounds

With further isolation, four purified compounds were obtained and they were analyzed by several of NMR techniques including ^{1}H-NMR and ^{13}C-NMR. Combining all the NMR spectra data, the four isolated compounds were finally recognized as camphor (0.46 g),[15] sabinene (0.22 g),[16–18] α-pinene (0.20 g)[19] and eucalyptol (0.15 g).[20]

Insecticidal activities of the essential oil

The essential oil of *A. blepharocalyx* rhizome parts showed contact toxicity against *L. serricorne* adults with an LD_{50} value of 15.02 μg adult$^{–1}$. Among the four main compounds, only camphor possessed stronger contact toxicity against *L. serricorne* (LD_{50} = 13.44 μg adult$^{–1}$), eucalyptol and sabinene exhibited the same level contact toxicity against *L. serricorne* (LD_{50} = 15.58 and 15.74 μg adult$^{–1}$) as the essential oil, while *α*-pinene showed weaker contact toxicity than the essential oil of *A. blepharocalyx* rhizomes (Table I). Compared with the famous botanical insecticide, pyrethrins, the essential oil was 63 times less active against *L. serricorne* adults because pyrethrins displayed LD_{50} value of 0.24 μg adult$^{–1}$ (Table I). However, the essential oil of *A. blepharocalyx* rhizome parts showed stronger contact toxicity against *L. serricorne* than the essential oil of *Zanthoxylum schinifolium* seeds (LD_{50} = 18.71 μg adult$^{–1}$).[21]

TABLE I. Toxicities of the essential oil of *Alpinia blepharocalyx* rhizome parts against *Lasioderma serricorne* adults; contact toxicity: LD_{50} / μg adult^{-1}; fumigant: LC_{50} / mg L^{-1} air

Toxicity	Treatment	Concentrations	LD_{50}/LC_{50}	95 % Fiducial interval	χ^2
Contact	*A. blepharocalyx*	2.20–10.00	15.02	12.88–17.21	9.660
	Camphor	1.97–10.00	13.44	10.39–16.07	15.38
	Eucalyptol	1.97–10.00	15.58	12.88–18.02	15.18
	Sabinene	1.97–10.00	15.74	10.33–20.70	7.31
	α-Pinene	5.93–30.00	77.28	69.02–87.30	14.71
	Pyrethrum	0.010–0.40	0.24	0.16–0.35	17.36
Fumi-gant	*A. blepharocalyx*	0.44–2.00	3.83	3.55–4.25	18.52
	Camphor	0.59–3.00	2.36	1.91–2.71	14.29
	Eucalyptol	0.99–5.00	5.18	4.63–5.70	16.79
	Sabinene	5.21–20.00	44.17	39.28–50.66	25.07
	α-Pinene	3.95–20.00	37.57	34.31–41.19	14.90
	Phosphine	7.20×10^{-3}–11.12×10^{-3}	9.23×10^{-3}	7.13×10^{-3}–11.37×10^{-3}	11.96

The essential oil of *A. blepharocalyx* rhizomes also possessed fumigant activity against *L. serricorne* with an LC_{50} value of 3.83 mg L$^{–1}$ air (Table I). In comparison, only camphor possessed stronger fumigant toxicity against *L. serricorne* (LC_{50} = 2.36 mg L$^{–1}$ air), eucalyptol exhibited the same level fumigant toxicity against *L. serricorne* (LC_{50} = 5.18 mg L$^{–1}$ air) as the essential oil, while sabinene and *α*-pinene showed weaker fumigant toxicity than the essential oil of *A. blepharocalyx* rhizomes (Table I). However, the currently used grain fumigant, phosphine has a fumigant activity against *L. serricorne* adults with an LC_{50} value of $9.23\times10^{–3}$ mg L$^{–1}$ air. The fumigant activity of the essential oil against the *L. serricorne* was thus many magnitudes lower than that of the commercial fumigants phosphine. Compared with the other essential oils investigated in previous studies, the essential oil of *A. blepharocalyx* rhizomes exhibited stronger fumigant toxicity against *L. serricorne*, than, *e.g.*, the essential oils of

Pistacia lentiscus (LC_{50} = 8.44 mg L^{-1} air), *Elsholtzia stauntonii* (LC_{50} = 10.99 mg L^{-1} air) and *Agastache foeniculum* (Lamiaceae) (LC_{50} = 21.57 mg L^{-1} air).[5,22,23]

In this work, the results suggested that among the four main components, camphor showed the strongest contact and fumigant toxicity against *L. serricorne*. In previous reports, the four components were demonstrated to possess insecticidal activities against several stored product insects, such as *Sitophilus zeamais*, *Tribolium castaneum*, *Leptinotarsa decemlineata*, and broadbean weevil.[24–28] The high volatility of these toxic compounds likely delivered fumigant toxicity by vapor action *via* the respiratory system, but further work is needed to confirm their exact mode of action.

A. blepharocalyx is one of the Dai drug types, the rhizome of which is used in the treatment of abdominal bloating and pain. Essential oil, which is a mixture of plant secondary metabolites, plays an important role in plant–insect interaction, and is commonly responsible for plant resistance to insects. The components of essential oil often biodegrade to non-toxic products and hence, they could be much safer insect control agents and more suitable for use in integrated pest management (IPM). As far as our literature survey could ascertain, no information concerning the insecticidal activity of the essential oil of *A. blepharocalyx* has been openly published. For this reason, in the present paper, the identification of the chemical components of *A. blepharocalyx* by a gas chromatography–mass spectroscopy (GC–MS) method was described, and the insecticidal property of the essential oil were evaluated against *L. serricorne*.

CONCLUSIONS

This study revealed that the essential oil of *A. blepharocalyx* rhizomes and its main compound camphor demonstrated significant insecticidal activity on *L. serricorne*. These findings also suggest that the bioactivities of the essential oil may be due to the bioactive compounds. Considering the currently used fumigants are synthetic insecticides, the essential oil of *A. blepharocalyx* rhizomes and camphor are quite promising, and they show potential for development as possible natural fumigants for the control of stored product insects. However, for the practical application of the essential oil and compounds as novel fumigants, further studies on the safety of the essential oil and compounds to humans and on development of formulation are necessary to improve the efficacy and stability, and to reduce cost.

SUPPLEMENTARY MATERIAL

Compounds identified in the essential oil of *Alpinia blepharocalyx* rhizome parts are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ХЕМИЈСКИ САСТАВ И ИНСЕКТИЦИДНА АКТИВНОСТ ЕТАРСКОГ УЉА РИЗОМА *Alpinia blepharocalyx* СПРАМ ДУВАНОВЕ БУБЕ *Lasioderma serricorne*

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Циљ рада је био одређивање хемијског састава и токсичности етарског уља ризома *Alpinia blepharocalyx* спрам дуванове бубе *Lasioderma serricorne* (Fabricius). Етарско уље из ризома *A. blepharocalyx* је добијено дестилацијом воденом паром и испитивано је методом GC–MS. Идентификовано је укупно 46 састојака. Главна једињења у етарском уљу *A. blepharocalyx* су била камфор (23,13 %), сабинен (11,27 %), α-пинен (9,81 %) и еукалиптол (8,86 %), а нађени су и камфен (8,05 %), силвестрен (5,61 %) и α-феландрен (5,00 %). Применом теста биоактивности идентификована су 4 активна састојка: камфор, сабинен, α-пинен и еукалиптол. Етарско уље *A. blepharocalyx* је испољило јаку контактну токсичност спрам дуванове бубе, уз LD_{50} вредност од 15,02 μg по одраслој јединки, као и јаку фумигантну токсичност, уз LC_{50} вредност од 3,83 mg L^{-1} ваздуха. Резултати су показали да етарско уље *A. blepharocalyx* може имати потенцијалну примену у заштити од инсеката током чувања дувана.

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