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RESEARCH ARTICLE

Larvicidal activity of major essential oils from stems of *Allium monanthum* Maxim. against *Aedes aegypti* L

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Abstract

The stems of *Allium monanthum* were extracted, and the major essential oil composition and larvicidal effects were studied. The analyses were conducted by gas chromatography and mass spectroscopy revealed that the essential oils of *A. monanthum* stems. The *A. monanthum* essential oil yield was 4.25%, and gas chromatography and mass spectroscopy analysis revealed that its major constituents were dimethyl trisulfide (23.21%), dimethyl tetrasulfide (11.24%) and methlyl propyl trisulfide (8.21%). The essential oil had a significant toxic effect against early fourth-stage larvae of *Aedes aegypti* L with an LC_{50} value of 23.14 ppm and an LC_{90} value of 36.31 ppm. Also, dimethyl trisulfide (≥95.0%), dimethyl tetrasulfide (≥95.0%) and methlyl propyl trisulfide (≥95.0%) were tested against the F_{21} laboratory strain of *A. aegypti*. Methlyl propyl trisulfide (≥95.0%) has good activity with an LC_{50} value of 19.38 ppm. Also, the above indicates that other major compounds may play a more important role in the toxicity of essential oil.

Keywords: *Aedes aegypti*, *Allium monanthum*, essential oils, larvicidal activity, methlyl propyl trisulfide

Introduction

Natural products are generally preferred because of their less harmful nature to non-target organisms[1]. Dengue virus infection is spread by the mosquito vector *Aedes aegypti*, and clinical manifestations of dengue fever vary from asymptomatic infection to serious disease. Symptoms include sudden onset of fever, retro-orbital headache, abnormal taste sensation, arthralgia, maculopapular rash, and anorexia[1]. They cause serious health problems to humans and present obstacles to the socioeconomic development of developing countries, particularly in the tropical region[2]. In the search for environmentally safe and relatively inexpensive methods to control mosquitoes, plant extracts have received much interest as potential bioactive agents against the mosquito larvae. Most mosquito control programs target the larval stage at their breeding sites with larvicides[3]. Since adulticides may reduce the adult population only temporarily[4]. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae. The mosquito *A. aegypti* is the world's most important vector of yellow fever and dengue viruses[5].

Essential oils are natural volatile substances found in a variety of plants. It is well known that plant-derived natural products are extensively used as biologically active compounds. Among them, essential oils were the first preservatives used by man, originally in their natural state within plant tissues and then as oils obtained by water distillation. Essential oils composed by isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of the smell found in the aromaticplants[6]. During our search for new types of natural products possessing an immunotoxicity activity from wild and cultivate plants, we investigated the essential oils from the leaves of *Zingiber officinale* Roscoe.

Allium monanthum Maxim (Liliaceae) is a perennial herb wildly distributed in field. *Allium* constituents have been shown to inhibit the covalent binding of the carcinogen, 7,12-dimethylbenza-anthracene, to DNA, an intracellular event that correlates with decreased mutagenesis and carcinogenesis[7]. Kowara group reported antiplatelet aggregation of *Allium* species has positive correlation with the content of organosulfuric

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substance[8]. Wu *et al*. searched the changes of the organosulfurics contents in shallots by processing[9]. Garlic, one of *Allium* species, has the activities of anti-platelet aggregation[10], antihepatotoxicity[11] and antitumor activity[12 13]. Some pharmacological properties of *A. monanthum* have been identified from 70% methanol extracts such as antioxidative activity[14]. Although little biological activity has been discovered from *A. monanthum,* details biological activity has not been fully characterized. The aim of this study was to investigate the immunotoxicity properties of the essential oils of *A. monanthum* stemes. To the best of our knowledge, this is the first report on the chemical composition and immunotoxicity activity of the essential oils of *A. monanthum* stems.

Experimental

Plant material and essential oils extraction

The *A. monanthum* stems provided to Korea local markets in May 2008 at Kyung-Ju, Kyungsangbukdo, South Korea. A voucher specimen (DKU-2008-02315) was deposited at herbarium, Jeonnam Institute of Natural Resources Research, Jangheung, South Korea, and identified by Dr. Hyung-In Moon, and subjected to hydrodistillation using a Clevenger type apparatus for 6 h. The essential oil was dried over anhydrous sodium sulphate and the purified essential oil was stored in an amber-coloured vial at 4°C until further use.

Chemicals

Dimethyl trisulfide (98.0%), dimethyl tetrasulfide (98.0%), allyl methyl disulfide (98.0%), dipropyl trisulfide (98.0%) and methlyl propyl trisulfide (98.0%) were purchased from Chemos GmbH (Regenstauf, Germany).

Gas chromatography–mass spectroscopy (GC–MS) analysis of the essential oils

GC–MS analysis of the essential oil was performed using a GC–MS spectrometer (QP 2010), equipped with a splitless injector. The components were separated on a 0.32 mm i.d. × 60 m DB-1 MS capillary column (Agilent Scientific) with a film thickness of 0.25 μm. The temperature program used for the analysis was as follows: The temperature of the injector was set at 300°C. The initial temperature was set at 80°C and held for 5 min, set at 5.0°C/min to reach 280°C, and held for 10 min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. One microlitre of the sample (diluted 1:10 with acetone) was injected with a split ratio of 1:100. The percent composition of the essential oil was calculated by comparing the areas of the GC peaks. The temperature of the ion source and of the injector was set at 200°C and 210°C, respectively. The interface was kept at 280°C and the mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 500 amu every 0.5 s. The different components of the essential oils were identified by comparing the mass spectra of each peak with those of authentic samples found in a library of mass spectra (The Wiley Registry of Mass Spectral Data, 7th edn.).

Immunotoxicity assay

The F[21] laboratory strain of *A. aegypti* was obtained in 2008 from the National Institute of Health, Seoul, South Korea. Adult female mosquitoes were maintained on a 10% sucrose solution, and anaesthetized mice were used for blood feeding the mosquitoes. Larvae were reared in plastic trays and fed a diet of chicken chow and yeast (8:2). Mosquitoes were maintained at 27 ± 2°C, 70 ± 5% relative humidity, and a photoperiod of 16L:8D. The immunotoxicity activity was analyzed according to the standard procedures recommended by the World Health Organisation[15]. The essential oil was dissolved in 1 ml of acetone and different concentrations were prepared (0, 25, 50, 75, and 100 ppm) using distilled water. Twenty larvae at the early fourth-stage were used in the immunotoxicity assay and five replicates were maintained for each concentration. The larval mortality was calculated after 24 h of exposure. The lethal concentrations LC_{50} and LC_{90}, the 95% confidence intervals, and the upper and lower confidence levels were calculated using profit analysis (SigmaPlot® software).

Results

The *A. monanthum* essential oil yielded 4.25% (v/w) essential oil with a foul odour. Table 1 lists its major chemical constituents, as identified by GC and GC/MS analyses. In their order of elution from the column, these compounds were dimethyl trisulfide (23.21%), dimethyl tetrasulfide (11.24%) and methlyl propyl trisulfide (8.21%). The immunotoxicity effects of the essential oil of the stems of *A. monanthum* are presented in Table 2. The oil had significant toxic effects against the larvae of *A. aegypti* with an LC_{50} value of 23.14 ppm and an LC_{90} value of 36.31. The control substance caused no mortality for the larvae. Also, dimethyl trisulfide (≥95.0%), dimethyl tetrasulfide (≥95.0%) and methlyl propyl trisulfide (≥95.0%) were tested against the F_{21} laboratory strain of *A. aegypti*. The current *A. monanthum* were tested for the first time against *A. aegypti*. However, dimethyl trisulfide

Table 1. Major essential oil constituents from stems of *Allium monanthum.*

No	Components	Peak area (%)
1	Allyl methyl disulfide	4.52
2	Dimethyl trisulfide	23.21
3	Allyl *cis*-1-propenyl disulfide	2.32
4	Allyl methyl trisulfide	3.21
5	Dimethyl tetrasulfide	11.24
6	Methlyl propyl trisulfide	8.21
7	Dipropyl trisulfide	3.02

Table 2. LC_{50} and LC_{90} values for major components against *Aedes aegypti*.

Essential oils/compounds	LC_{50} (ppm)	LC_{90} (ppm)
Allium monanthum	23.14	36.31
Dimethyl trisulfide	>200	>200
Dimethyl tetrasulfide	>200	>200
Methlyl propyl trisulfide	19.38	58.42
Allyl methyl disulfide	>200	>200
Dipropyl trisulfide	>200	>200
1,8-cineole[a]	>200	>200

[a]Negative control.

(≥95.0%) and dimethyl tetrasulfide (≥95.0%) have no activity. Methlyl propyl trisulfide (≥95.0%) has good activity. Methlyl propyl trisulfide (≥95.0%) was the most toxic among the major components, with an LC_{50} value near 19.38 ppm. 1,8-cineole (negative control) revealed no toxicity. The immunotoxicity effects are summarized in Table 2.

Discussion

In general, plant essential oils have been recognized as an important natural source for insecticides[16 17]. The differences in the toxicity of essential oils against different mosquito species are well known[18] and are due to qualitative and quantitative variations of the components. Recently the clinical use of essential oils has expanded worldwide also including therapy against various kinds of inflammatory diseases, such as allergy, immuntoxicity, rheumatism and arthritis. These activities have mainly been recognized through clinical experience, but there have been relatively little scientific studies on biological actions of these natural essential oil extracts. For instance, Lee *et al*. described immunotoxicity activity of 2,6,10,15-tetrame-heptadecane from the essential oils of *Clerodendrum trichotomum* Thunb. against *A. aegypti* L. The chemical components in oil extracted from the leaves of *C. trichotomum* after identification of the chemical constituents with the help of GC, GC–MS are recently reported[19] Lee *et al.* reported that *Filipendula glaberrima* growing in the middle region in South Korea contained beta-farnesol (2.96%), l-alpha-terpineol (2.43%), benzenemethanol (2.87%), (Z)-3-hexen-1-ol (5.23%), and 2,6-bis(1,1-dimethylethyl)-4-methylphenol (1.91%) as major components. The immunotoxicity activities of the oil from *F. glaberrima* obviously increase with increasing concentration of essential oils[20]. Park *et al.* described composition and immunotoxicity activity of the major essential oil of *Angelica purpuraefolia* Chung against *A. aegypti* L[21]. The findings of the present study indicate that the essential oil extracted from the leaves of *Allium victorialis* could be studied as a potential natural immunotoxicity effects. *A. monanthum* essential oils are present only in minute amounts. They have immunotoxicity activity *in vitro,* which has not been verified in animals or humans. Because minute amounts of single compounds within *A. monanthum* has been shown to account for its clinical effects, most herbalists now conclude that it is a combination of ingredients, rather than a sufficient single ingredient, that accounts for *A. monanthum* medicinal effects. So, field evaluation and further investigations on the effects on non-target organisms are necessary.

Declaration of interest

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References

1. World Health Organization. Dengue haemorrhagicfever: diagnosis, treatment, prevention and control. 2nd edn. Geneva: WHO, 1997.
2. Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of Blumea mollis (D. Don) Merr. against Culex quinquefasciatus. Parasitol Res 2008;103:959–962.
3. Knio KM, Usta J, Dagher S, Zournajian H, Kreydiyyeh S. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, Ochlerotatus caspius. Bioresour Technol 2008;99:763–768.
4. El Hag EA, Rahman Abd-El., El-Nadi H, Zaitoon AA. Effects of methanolic extracts of neem seeds on egg hatchability and larval development of Culex pipiens mosquitoes. Indian Vet J 2001;78;199–201.
5. Scott TW, Chow E, Strickman D, Kittayapong P, Wirtz RA, Lorenz LH et al. Blood-feeding patterns of Aedes aegypti (Diptera: Culicidae) collected in a rural Thai village. J Med Entomol 1993;30:922–927.
6. Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ. Bioactivity of selected plant essential oils against the yellow fever mosquito Aedes aegypti larvae. Bioresour Technol 2003;89:99–102.
7. Moon KS. Usage and Components of Herbal Medicine, Scientific Encyclopedia Co., Pyongyang, 1984; pp. 671–672.
8. Wijaya CH, Muchtani D, Lalel HJ, Zakaria F, Kowara S. Antiplatelet aggregation potencies of some Allium spp. grown in Indonesia. Nat Prod Sci 1996;2;37–42.
9. Wu JL, Chou CC, Chen MH, Wu CM. Volatile flavor compounds from shallots. J Food Sci 1982; 47;606–608.
10. Liakopoulou-Kyriakides M, Sinakos Z, Kyriakidids DA. Identification of alliin, a constituent ofAIlium cepa with an inhibitory effect on platelet aggregation. Phytochemistry 1985; 24;600–601.
11. Hikino H, Tohkin M, Kiso Y, Namiki T, Nishimura S, Takeyama K. Antihepatotoxic actions of Allium sativum bulbs. Planta Med 1986;163–168.
12. Siegers CP, Steffen B, Röbke A, Pentz R. The effects of garlic preparations against human tumor cell proliferation. Phytomedicine 1999;6:7–11.
13. Han SS, Lo SC, Choi YW, Kim JH, Baek SH. Antioxidant activity of crude extract and pure compounds of Acer ginnala Max. Bull. Korean Chem Soc 2004;25;389–391.
14. Shirataki Y, Motohashi N, Tani S, Sunaga K, Sakagami H, Satoh K et al. Antioxidative activity of Allium victorialis L. extracts. Anticancer Res 2001;21:3331–3339.
15. World Health Organisation. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO-VBC 1981;81;807–962.
16. Gbolade AA, Oyedele AO, Sosan MB, Adewayin FB, Soyela OL. Mosquito repellent activities of essential oils from two Nigerian Ocimum species. J Trop Med Plants 2000;1;146–148.
17. Adebayo TA, Gbolade AA, Olaifa JI. Comparative study of toxicity of essential oils to larvae of three mosquito species. Niger J Nat Prod Med 1999;3;74–76.

18. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 1991;7:210–237.
19. Lee SJ, Moon HI. Immunotoxicity activity of 2,6,10,15-tetrame-heptadecane from the essential oils of Clerodendron trichotomum Thunb. against Aedes aegypti L. Immunopharmacol Immunotoxicol 2010;32;705–707.
20. Lee SJ, Moon HI. Immunotoxicity activity of the major essential oil of *Filipendula glaberrima* against Aedes aegypti L. Immunopharmacol Immunotoxicol 2010;32:617–619.
21. Park YJ, Chung IM, Moon HI. Effects of immunotoxic activity of the major essential oil of *Angelica purpuraefolia* Chung against Aedes aegypti L. Immunopharmacol Immunotoxicol 2010;32:611–613.