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Chemical Composition and Antifungal Activity of Essential Oil from *Cymbopogon nardus* (Citronella Grass)

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Abstract

The objective of this study was to elucidate the chemical composition of essential oil from *Cymbopogon nardus* (citronella oil) and its antifungal activity. Chemical composition of the citronella oil was determined by capillary gas chromatography (GC) and GC/ mass spectrometry. Major constituents of the oil were geraniol (35.7% of total volatiles), *trans*-citral (22.7%), *cis*-citral (14.2%), geranyl acetate (9.7%), citronellal (5.8%) and citronellol (4.6%). The antifungal assay using the vapor-agar contact method showed that the crude essential oil markedly suppressed the growth of several species of *Aspergillus*, *Penicillium* and *Eurotium* at a dose of 250 mg/L in air. The most active compounds among the 16 examined volatiles, consisting of 6 major constituents of the essential oil and 10 other related monoterpenes were citronellal and linalool. Citronellal and linalool completely inhibited the growth of all tested fungal strains at a dose of 112 mg/L. Their minimum inhibitory doses ranged from 14 to 56 mg/L. The α- and β- pinenes showed an inhibitory activity against some fungi, whereas the other 8 volatile compounds lacked this property.

Discipline: Postharvest technology
Additional key words: antifungal activity, *Cymbopogon nardus*, citronellal, linalool, mycotoxin, vapor contact, *Aspergillus*

Introduction

Substantial amounts of stored food products are attacked by fungi, insects and animals worldwide. In particular, food products in developing countries located in tropical and subtropical regions experience serious damage, leading to economic losses and health hazard as a result of mycotoxin production. Synthetic chemicals and fumigants have been widely used for preventing quality deterioration of stored products. However, these agents will be phased out in the near future due to their potential adverse impact on the environment. Therefore, biodegradable alternatives should be developed worldwide for reducing postharvest losses. In fact, selected plants and their essential oils have been evaluated as natural sources for controlling storage fungi. Some of the aromatic plants which are widely distributed in the tropical zone and exhibit fungicidal activities, have traditionally been used as flavoring agents in native dishes, and as incense, insect repellents and folk medicine[2,6].

Cymbopogon nardus Randle *ExG* is a perennial grass cultivated in Southeast Asia. The essential oil from *C. nardus* is known as citronella oil, and has been traditionally used as mosquito repellent, household fumigant, or fragrance agent in food commodities, soaps and

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cosmetics[2,3,6]. Although some previous studies using the disc diffusion method had revealed that citronella oil exhibited antibacterial and antifungal activities, the active components were not identified[1,9]. In this study, the antifungal activity of citronella oil, its components and other volatiles were investigated using the vapor-agar contact method to develop a more practical approach for controlling fungi in storage facilities for food products.

Materials and methods

1. Plant material and steam distillation

Cymbopogon nardus Randle *ExG* was collected around Bangkok, Thailand, in August 2000. A representative accession (TNS 9515379) was deposited at Tsukuba Botanical Garden, National Science Museum, Japan. Aerial parts (5.0 kg) were steam-distilled for 1 h with 10 volumes of water. Essential oil was then extracted with diethylether which was evaporated before antifungal experiments were conducted.

2. Gas chromatography / mass spectrometry analysis

The capillary gas chromatography (GC) and GC/mass spectrometry analyses of the essential oil were carried out using a model Hewlett-Packard (HP) 5980 series 2 equipped with 5989A at an ionization energy of 70 eV. The temperature program was 70ºC for 2 min, increasing up to 180ºC at 5ºC/min. The components were identified by direct comparison of their Kovat's index[7] on polar and apolar columns and mass spectra with authentic preparations.

3. Determination of antifungal activity

Nine fungal strains, *Aspergillus candidus* IFO 8816, *A. flavus* IFO 4186, *A. versicolor* IFO 31806, *Eurotium amstelodami* IFO 5721, *E. chevalieri* IFO 4086, *Penicillium adametzii* IFO 7223, *P. citrinum* IFO 4631, *P. griseofulvum* IFO 7011, and *P. islandicum* IFO 5234, supplied by the Institute of Fermentation Osaka (IFO) were used in this study. These fungi which produce mycotoxins are often detected in rice exported from Thailand[11]. Volatile compounds (analytical grade) were purchased from Wako Pure Chemical (Osaka, Japan). Antifungal activity was determined by the vapor-agar contact method previously described by Sekiyama et al.[10] with a slight modification as follows. Fungi were cultured on potato dextrose agar (PDA) medium at 27ºC for a week before the experiment. Fungal spores (approximately 1.5×10^3) were then inoculated in the center of PDA plates (40 mm diameter) which were aseptically placed in a chamber (capacity, 300 mL) without lids. Tested volatile compounds were introduced into the chambers followed by proper sealing (Fig. 1). Incubation in the chambers was performed at 27ºC for 3 to 5 days, the period required for colony formation (approximately 8 mm in diameter) on agar plates in the control chambers. The inhibitory activity was evaluated by measuring the diameter of colonies formed by the tested fungal strains. The minimum inhibitory dose (MID) was defined as the lowest concentration (mg/L in air) of volatile compounds which inhibited colony formation of test fungi by 50%.

Results and discussion

C. nardus yielded 0.14% v/w of a colorless essential oil with a distinct aroma. The capillary GC and GC-MS analyses indicated that monoterpenes predominated in the oil (Table 1). The major constituents of the oil were geraniol (35.7% of total volatiles), *trans*-citral (22.7%), *cis*-citral (14.2%), geranyl acetate (9.7%), citronellal (5.8%) and citronellol (4.6%). The composition of the essential oil in this study was significantly different from that previously reported by Mahalwal and Ali[8]. The major components of the essential oil from *C. nardus* cultivated in India were citronellal (29.7%), geraniol (24.2%), γ-terpineol (9.2%) and *cis*-sabinene hydrate

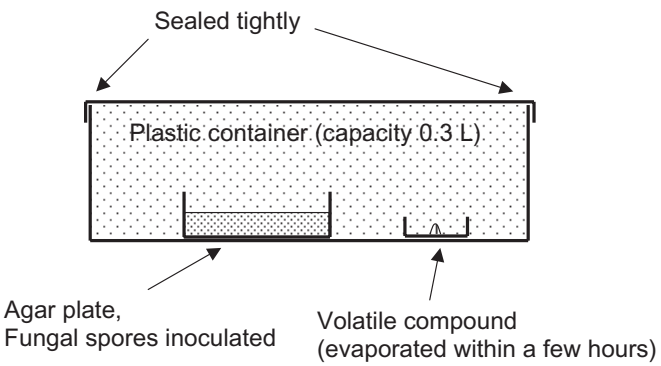

Fig. 1. Determination of antifungal activity using vapor-agar contact method

Table 1. Chemical composition of essential oil from *C. nardus*

Compounds[a)]	Percentage of total oil	Retention index	
		apolar[b)]	polar[c)]
Linalool	1.3	1,094	1,537
Citronellal	5.8	1,148	1,465
Citronellol	4.6	1,224	1,755
cis-Citral	14.2	1,238	1,582
Geraniol	35.7	1,253	1,834
trans-Citral	22.7	1,268	1,613
Geranyl acetate	9.7	1,380	1,745
β-Caryophyllene	0.8	1,421	1,572
Identified compounds	94.8		

a): Compounds are listed in the order of elution on MPS-5 column.
b): Retention index on apolar MPS-5 column (Quadrex).
c): Retention index on polar PEG20M column (Quadrex).

(3.8%)[8]. It is generally recognized that there are several chemotypes (marker compounds) in the same plant, such as those of *Thymus vulgaris*[4,5]. It was reported that the citronellal content varied among the cultivated varieties of citronella grass. Essential oil from Maha Pengiri (Sri Lanka and Indonesia) contained 40.5–60.7% of citronellal, whereas the oil from *C. nardus* var. *confertiflorus* (India) contained much smaller amounts of citronellal (17.2–33.2%)[8]. Additionally, the composition of essential oils is affected by many factors, including the cultivation conditions of the plants and isolation techniques[5].

The essential oil from *C. nardus* completely inhibited all 9 tested fungal strains at a dose of 250 mg/L. However, no inhibitory effect was detected by the agar dilution method in this study at the same dose (data not shown). As was the case in previous reports, the agar dilution method and vapor phase method gave different MID values for the same essential oil[5]; *A. niger* was inhibited by a higher MID value (800 mg/L) of citronella oil using the agar dilution method[4]. Moreover, the vapor phase of Hyssop oil was inactive against *Sarcina ureae*, while the oil showed an activity when the paper disk

Table 2. Antifungal activity of volatile compounds selected by gaseous contact

Volatile compounds (112 mg/L)	Growth inhibition								
	A. c.	A. f.	A. v.	E. a.	E. c.	P. a.	P. c.	P. g.	P. i.
Citronellyl acetate	–	–	–	–	–	–	–	–	–
Geranyl acetate	–	–	–	–	–	–	–	–	–
α-Terpineol	–	–	–	–	–	–	–	–	+
α-Terpinene	–	–	–	–	+	–	–	–	–
Limonene	–	–	–	–	–	–	–	–	–
Isoeugenol	–	–	–	–	–	–	–	–	–
Citronellal	++ (28)	++ (56)	++ (28)	++ (28)	++ (14)	++ (56)	++ (28)	++ (56)	++ (14)
Citronellol	–	–	–	–	+	–	–	–	–
Linalool	++ (28)	++ (56)	++ (56)	++ (28)	++ (28)	++ (56)	++ (28)	++ (56)	++ (28)
Geraniol	–	–	–	–	–	–	–	–	+
Citral	–	–	–	–	+	–	–	–	+
Nerol	–	–	–	–	–	–	–	–	–
Menthone	+	–	–	++	++	+	+	++	+
α-Pinene	++	–	++	–	++	–	–	++	+
β-Pinene	++	–	++	–	++	–	–	++	+
Myrcene	–	–	–	–	–	–	–	–	–

A. c.: *Aspergillus candidus*, A. f.: *A. flavus*, A. v.: *A. versicolor*, E. a.: *Eurotium amstelodami*, E. c.: *E. chevalieri*, P. a.: *Penicillium adametzii*, P. c.: *P. citrinum*, P. g.: *P. griseofulvum*, P. i.: *P. islandicum*.
++: potent activity (colony size in diameter < 1 mm), +: moderate activity (1–4 mm), –: weak or no detectable activity (> 4 mm). Numbers in parentheses refer to vapor MID values (mg/L in air).

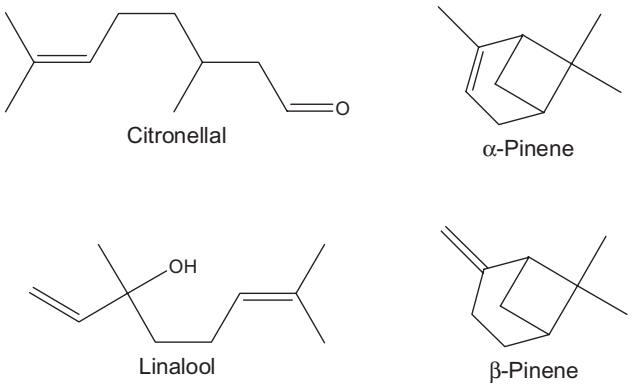

Fig. 2. Volatile compounds showing antifungal effects

method was used[5].

Among the 16 examined volatiles, linalool and citronellal (Fig. 2) were the most active compounds against fungi with MID values ranging from 14 to 56 ppm (Table 2). However, three other major constituents of citronella oil, geraniol, citral and geranyl acetate, did not show any activity or showed a very weak activity at 112 mg/L (Table 2). These results suggested that linalool and citronellal contributed significantly to the total antifungal activity of citronella oil used in the present study. De Billerbeck et al. previously reported that citral accounted for > 70% of the antifungal activity of *C. citratus* (lemongrass) essential oil, and was less active against *A. niger* than the antifungal constituent of citronella oil[4]. Another interesting point in the present study was that menthone inhibited two *Eurotium* strains and *P. griseofulvum*, but did not show any activity against *A. flavus* and *A. versicolor*. The α- and β-pinenes (Fig. 2) displayed a potent activity against *A. candidus*, *A. versicolor*, *E. chevalieri* and *P. griseofulvum*.

C. nardus, a plant growing wild in Thailand and in other Asian countries, could become a renewable source for natural fungicides. Its essential oil, especially the active constituents (citronellal and linalool), was a potent inhibitor (via vapor phase) of various fungi at ambient temperatures. The compounds with lower MID values against different fungal genera and species could be used as natural alternatives for synthetic fumigants to protect stored food products.

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