Original Article

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Anti-*Candida* and radical scavenging activities of essential oils and oleoresins of *Zingiber officinale* Roscoe and essential oils of other plants belonging to the family Zingiberaceae

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ABSTRACT: Essential oils of young and mature rhizomes, air-dried and steamed rhizomes, and seed rhizomes of *Zingiber officinale* Roscoe (ginger) were prepared, and their inhibition of filamentation by *Candida albicans* was examined. Ginger essential oils, and particularly those from seed and air-dried rhizomes, had potent inhibitory activity compared to ginger oleoresins obtained by ethanol and hypercritical carbon dioxide extraction and essential oils of 5 other plants in the family Zingiberaceae. Of the constituents, [6]-shogaol was most active against filament formation and growth of *C. albicans*, followed by citral and [6]-gingerol. Ginger oleoresin, and especially that obtained by ethanol extraction, with a high [6]-gingerol content exhibited potent scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radicals in comparison to essential oils of ginger and other Zingiberaceae plants.

Keywords: Essential oil, oleoresin, *Z. officinale*, [6]-gingerol, [6]-shogaol, citral, *Candida albicans*, radical scavenging activity

1. Introduction

Oral candidiasis is common in patients with AIDS, hypo-salvation, and diabetes mellitus and in individuals with poor oral hygiene. Chemotherapy is usually effective at treating oral candidiasis but causes new problems clinically, such as frequent recurrence of the disease and the emergence of drug-resistant *Candida* strains. Therefore, new therapies such as oral administration of edible materials that have anti-*Candida* activity should be developed.

Essential oil from rhizomes of *Zingiber officinale* Roscoe (abbreviated as ginger below) has been the subject of numerous studies worldwide. The oil has been reported to have stimulating action (*1*), anti-spasmodic action (*2*), immunomodulation (*3,4*), anti-inflammatory action (*5*), and other types of activity. However, there are relatively few reports on the oil's antimicrobial action, which is probably because of the weakness of that action (*6*). Most recently, essential oil of *Alpinia galanga*, a plant of the Zingiberaceae family, has been reported to have strong anti-microbial activity (*7*).

The current study focused on the anti-*Candida* activity of ginger essential oil. Ginger oils were prepared using young and mature rhizomes and air-dried and steamed rhizomes, and their inhibition of filamentation and growth of *C. albicans* was determined. The anti-*Candida* activity of ginger oils was compared to that of 2 ginger oleoresins obtained by ethanol and hypercritical carbon dioxide (CO_2) extraction and 5 other oils belonging to the family Zingiberaceae. These oils were plai (rhizomes of *Z. cassumunar* Roxb.), ginger lily (flower and leaves of *Hedychium coronarium* Koenig), kapurkachri (rhizomes of *H. spicatum*), gettou (leaves of *Alpinia zerumbet* B.L. Burtt et R.M. Sm.), and myoga (flower buds of *Z. mioga*). Plai is native to Indonesia, Thailand, and India and is used in folk medicine. Ginger lily is an erect shrub grown in the tropical and semi-tropical portions of India. It is cultivated for ornamental use and also for Chinese medicine. Kapurkachri is grown in mountainous areas of India and southern China and is used in Chinese medicine. Gettou is grown in tropical and subtropical regions and is known as wild ginger or bitter ginger. It is cultivated in Okinawa and the Ogasawara Islands of Japan for decoration. Myoga is a traditional Japanese vegetable with edible flower buds produced in the summer/autumn. Young ginger rhizomes are called "hashouga" in Japanese and are eaten raw in Japan.

The current study sought to evaluate the anti-*Candida* activity of ginger essential oil, and particularly its inhibition of filament formation by *C. albicans*, and to evaluate its scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by comparing ginger essential oil to ginger oleoresins and other related essential oils.

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2. Materials and Methods

2.1. *Essential oils, oleoresins, and chemicals*

Young and mature ginger oils were obtained by steam distillation of fresh rhizomes collected from a farm in the Town of Shimanto, Kochi Prefecture, Japan in August and November 2010, along with the seed rhizome (propagation rhizome) connected to the fresh rhizome underground. Air-dried ginger oil was prepared from mature rhizomes dried for a week in the shade, and steamed oil was obtained from mature rhizomes after steam-heating for 2 h followed by drying. Myoga oil was prepared from fresh flower buds of myoga cultivated in the City of Suzaki, Kochi Prefecture, Japan in August 2009. Details of steam distillation were described in a separate paper (Inouye *et al*., submitted for publication).

Ethanol oleoresin was prepared by two cycles of extraction of fresh mature rhizomes (231 g) with ethanol (300 mL). The first ethanol extract contained much of the rhizome's water content, and most constituents were extracted with the second extraction. Both extracts were combined and evaporated under reduced pressure to dryness to yield 290 mg of resinous oleoresin.

Plai oil prepared in Indonesia and ginger lily, kapurkachri oils, and CO_2 oleoresin of ginger rhizomes prepared in India were supplied by Phyto-aroma Laboratories, Yokohama, Japan. Gettou oil prepared in Okinawa was supplied by Yumejin Limited, Okinawa, Japan. [6]-Gingerol, [6]-shogaol, citral, β-bisabolene and dextrose were purchased from Wako Pure Chemical Co., Ltd., Osaka, Japan. RPMI-1640 medium containing phenol red was obtained from Sigma Chemical, Ltd. (St. Louis, MO, USA). Calf serum was obtained from Thermo Trace, Ltd. (Melbourne, Australia). Bacto™ Yeast Extract and Bacto™ Peptone were purchased from Becton-Dickinson & Company (MD, USA). Alamar Blue was obtained from Trek Diagnostic Systems, Inc. (Cleveland, OH, USA).

2.2. *Compositional analysis*

GC/MS analysis of essential oils and oleoresins was performed using a Shimadzu QP-2010 instrument (Shimadzu, Kyoto, Japan) with a TC-5 column. Quantitative analysis was carried out using a Model 353B GC instrument equipped with a TC-5 column and a hydrogen flame detector. GC conditions were as previously described (*8*).

HPLC analysis was carried out using a Waters Alliance instrument (Tokyo, Japan) coupled to a Waters ZQ MS ESI instrument with a mobile phase of 50 mM ammonium acetate (A), acetonitrile (B), and water (C) at a ratio of A:B:C = 10:10:80 (0-10 min, linear gradient flow) and 10:10:0 (10-20 min, fixed flow). The flow rate was 0.4 mL/min at 30°C, and peaks were detected using UV absorption at 280 nm.

[6]-Gingerol content was determined by HPLC using an authentic sample as an external standard and absorbance at 280 nm or MS intensity at m/z 293. The correlation coefficient was 0.9999 for 280 nm and 0.9983 for m/z 293.

2.3. *Assay of the inhibition of filament formation and growth of Candida albicans*

C. albicans TIMM1678, a stock culture of Teikyo University Institute of Medical Institute, was used in an inhibition assay. An assay of filamentation inhibition was carried out according to the method used by Abe *et al*. (*9*) and is briefly described below.

An essential oil or oleoresin was dissolved in DMSO in a series of two-fold dilutions. Each DMSO solution was diluted with RPMI-1640 medium so as to obtain a 0.25% DMSO concentration for the final assay medium. A sample (100 μL) and a fungal solution (500 cells/mL, 100 μL) suspended in RPMI-1640 containing 2.0% calf serum were mixed in a 96-well flat microplate. The mixture was cultured at 37°C for 16 h. After the yeast form was washed out with distilled water, the filamentous form was stained with 0.02% crystal violet (10 μL) for 15 min. The dye adsorbed on the filamentous cells was eluted with isopropanol (150 μL) containing 0.25% dodecyl sulfate and 0.04 N hydrochloric acid, and the extract's absorbance at 620 nm was recorded. The rate of inhibition of filamentous formation was expressed as follows:

Rate of inhibition (%) = $(1 - OD_{sample}/OD_{control}) \times 100$, where OD_{sample} is the optical density of a sample solution, and $OD_{control}$ is the optical density of a control solution.

The assay of growth inhibition was carried out using the same RPMI-1640 medium used for the assay of filamentation inhibition, and YPD medium (yeast 1%, peptone 2% and dextrose 5%) as previously reported (*10*). A sample solution in a series of two-fold dilutions containing less than 0.5% DMSO (100 μL) and a fungal suspension (2×10^3 cells/mL) in medium (100 μL) were mixed in the wells of a microplate. Alamar Blue (10 μL) was added when RPMI-1640 was used. The microplate was incubated at 30°C, and growth was stopped when a control well turned red in the event RPMI-1640 was used or absorbance at 620 nm exceeded 0.2 in of the event YPD was used. The minimum concentration needed for the well to remain blue was defined as the MIC. *C. albicans* TIMM1678 grows in both filamentous and yeast forms in RPMI-1640 medium, and turbidimetry was difficult. However, *C. albicans* TIMM1678 grows in the yeast form in YPD medium, and turbidimetry was used when that medium was used. When absorbance at 620 nm was 50% of the control, the IC_{50} value (minimum concentration to reduce the growth 50%) was obtained. At the same time,

inhibition percentage at 4,000 µg/mL was recorded since the IC_{50} value could not be obtained for many oils because of their weak activity.

2.4. *DPPH radical scavenging assay*

The detailed procedures for an assay of DPPH radical scavenging have previously been reported (*11*) and briefly described here. An essential oil or a pure compound was dissolved in ethanol in a series of two-fold dilutions, starting at 4,000 µg/mL to 3.9 µg/mL. Eugenol and α-tochopherol were used as positive controls. Each ethanol solution (75 µL) was added to 150 µL of 100 mM (0.012%) tri-(hydroxymethyl)-aminomethane hydrochloride buffer (pH 6.5) in 50% ethanol in wells of a microplate (Multi Well Plate, Sumitomo Bakelite Co., Ltd., Tokyo, Japan). A DPPH solution (0.2 mg/mL) in ethanol (75 µL) was added to the mixture, and the microplate was covered with a plastic film (Sumitomo Bakelite Co., Ltd.), shaken vigorously with an agitator, and allowed to stand at room temperature in a dark room. The absorbance at 540 nm was measured after 6 h using a multiscan photometer (Multiskan FC (Skanlt 3.1), Thermo Fisher Scientific Ltd., Yokohama, Japan). Relative scavenging activity (RSA) was calculated using the following equation:

RSA (%) = (1 – As/Ac) × 100, where Ac is the absorbance of a control sample, and As is the absorbance of a test sample. The EC_{50} value (effective concentration to decrease the initial DPPH concentration by 50%) was calculated.

3. Results

Table 1 shows the composition of essential oils, oleoresins, and ethyl acetate extracts from various forms of ginger rhizomes. The major constituent of essential oils was citral, which increased in the oils obtained from air-dried rhizomes and seed rhizomes. A particularly high percentage (70%) was noted in air-dried specimens. In contrast, young rhizomes had the lowest content of citral and highest content of geranyl

Table 1. Composition of essential oils, oleoresins, and solvent extracts of ginger as determined by GC

Table 1A. Compositions of essential oils

Constituent[a]	Ginger oil (fresh rhizomes)			Ginger oil (dry rhizomes)	
	Young (%)	Mature (%)	Seed (%)	Air-dried (%)	Steamed (%)
Zingiberene	6.3	17.1	14.2	3.8	10.3
Ar-curcumene	1.3	2.8	5.2	1.9	18.1
β-Bisabolene	4.2	6.8	7.2	1.8	18.7
Farnesene	0.3	0.5	0.7	0.2	7.0
β-Sesquiphellandrene	2.8	5.9	6.4	2.1	14.1
Citral[b]	12.6	24.9	46.0	70.5	7.0
Geraniol	8.6	4.7	2.4	1.7	1.9
Geranyl acetate	45.0	6.7	0.2	0.1	7.6
1,8-Cineole	1.6	6.1	1.7	0.8	-
Camphene	0.1	3.1	0.6	-	0.8
Sabinene	0.7	6.3	1.7	0.5	-
[6]-Gingerol	-	-	-	-	-
[6]-Shogaol	-	-	-	-	-

Table 1B. Composition of oleoresins and solvent extracts

Constituent[a]	Oleoresin		Ethyl acetate extract		
	EtOH[c] (%)	CO_2[d] (%)	Young (%)	Mature (%)	Steamed (%)
Zingiberene	11.8	27.1	28.8	26.2	30.4
Ar-curcumene	4.2	15.1	3.4	6.0	9.7
Bisabolene	7.1	11.8	11.7	10.8	20.2
Farnesene	0.4	3.1	0.3	1.3	3.3
β-Sesquiphellandrene	8.0	14.0	10.8	10.3	14.8
Citral[b]	-	-	0.6	5.1	-
Geraniol	-	-	2.8	-	-
Geranyl acetate	-	-	8.9	0.2	1.5
1,8-Cineole	-	1.8	2.3	3.1	0.2
Camphene	-	2.1	0.2	5.3	1.1
Sabinene	-	2.3	0.6	4.8	1.1
[6]-Gingerol	19.4	1.6	3.6	2.9	1.0
[6]-Shogaol	14.0	0.6	-	-	6.5

[a] Listed are constituents at levels of more than 3.0% in at least one oil, oleoresin, or extract; [b] Combined content from geranial and neral; [c] Oleoresin obtained by ethanol extraction; [d] Oleoresin obtained by supercritical carbon dioxide extraction.

acetate. The lowest citral content was found in steam-heated rhizomes.

The next major constituents were sesquiterpenes consisting of zingiberene, β-sesquiphellandrene, β-bisabolene, and ar-curcumene while the sesquiterpene farnesene was a minor constituent. The steam-heated sample had a higher content of ar-curcumene. 1,8-Cineole, camphene, and sabinene were present as minor constituents.

A similar composition was noted for oleoresins and ethyl acetate extracts of ginger, with had five sesquiterpenes as major constituents. 1,8-Cineole, camphene, and sabinene were found to be minor constituents except in ethanol oleoresin, in which no monoterpene hydrocarbon was detected. However, oleoresins and ethyl acetate extracts had quite a low citral, geraniol, and geranyl acetate content; the extract of young rhizomes, however, had a relatively high geranyl acetate content.

Gingerol and shogaol, two major pungent principals of ginger rhizomes, were not present in the essential oils but were present in the oleoresins and solvent extracts according to GC analysis. [6]-Gingerol was found to be a major constituent of ethanol oleoresin while [6]-shogaol was a minor constituent. Although ethanol oleoresin had a high percentage of [6]-gingerol, it contained many other compounds with a high molecular weight, such as stigmasta-4,22-dien-3-ol (C_{29}), clionasterol (C_{29}), and other unidentified compounds. These less volatile constituents were detected by the QP-2010 GC/MS instrument but not by the 353B GC instrument. These high molecular compounds were not present in CO_2 oleoresin.

Since some gingerol was converted to shogaol due to thermal decomposition during GC, HPLC analysis was performed to determine the [6]-gingerol content and relative ratio of gingerol and shogaol. As shown in Table 2, [6]-gingerol was found to be a major constituent of ethanol oleoresin while [8]- and [10]-gingerols were minor constituents. [6]-Shogaol content was 1/3 of the [6]-gingerol content in ethanol oleoresin. CO_2 oleoresin prepared in India had a composition of gingerol and shogaol similar to that of ethanol oleoresin, although the concentration of [6]-gingerol was lower. Ethyl acetate extract of young and mature rhizomes contained only gingerols and no shogaol, but the [6]-shogaol content increased markedly in steam-heated rhizomes.

Table 3 shows the composition of the reference oils. Plai essential oil mainly consisted of sabinene (40.1%), terpinen-4-ol (24.8%), β-terpinene, *p*-cymene, γ-terpinene, and β-sesquiphellandrene. 1,4-Bis-methoxy-triquinacene was detected only by the QP-2010 GC/MS instrument.

The flower essential oil of ginger lily consisted mainly of sesquiterpenes such as 6-ethenyl-3-

Table 2. Composition of gingerol and shogaol in ginger oleoresins and ethyl acetate extracts as determined by HPLC

Source	Relative percentage (concentration, μg/mg)[a]			
	[6]-Gingerol	[6]-Shogaol	[8]-Gingerol	[10]-Gingerol
Ethanol oleoresin	46.9% (256.8)	15.1%	7.7%	12.2%
CO_2 oleoresin	3.2% (115.5)	12.9%	4.5%	7.3%
Young rhizome extract[b]	77.9%	-	22.1%	-
Fresh rhizome extract[c]	65.2%	-	6.4%	10.9%
Steamed rhizome extract[d]	46.1%	25.6%	7.1%	8.6%

[a] Absorbance at 280 nm. [6]-gingerol content determined using m/z 293 was 284.2 μg/mg in ethanol oleoresin and 156.6 μg/mg in CO_2 oleoresin; [b] Ethyl acetate-ethanol extract from fresh young rhizomes; [c] Ethyl acetate extract from fresh mature rhizomes; [d] Ethyl acetate extract from steam-heated and dried mature rhizomes.

Table 3. Composition of essential oils from plants belonging to the family Zingiberaceae except for ginger

Essential oil	Constituent[a]
Plai (rhizomes)	Sabinene (40.1%), terpinen-4-ol (24.8%), β-terpinene (7.9%), *p*-cymene (4.5%), γ-terpinene (4.1%), β-sesquiphellanderene (3.0%), 1,4-bismethoxy-triquinacene[b]
Ginger lily (flowers, leaves)	Methoxy-sesquiterpene[c] (32.4%), camphor (13.7%), germacra-trien-6-one (8.4%), 1,8-cineole (6.0%), furanosesquiterpene[d] (6.1%), β-elemene (5.4%), camphene (3.9%), borneol (3.5%)
Kapukachri (rhizomes)	1,8-Cineole (25.4%), agarospirol (10.6%), β-eudesmol (9.9%), cadina-1(10),4-diene (7.5%), β-elemol (5.8%), α-eudesmol (5.2%), cadina-1(11),4-diene (4.3%), α-cadinol (4.3%), cubenol (4.1%)
Gettou (leaves)	β-Phellandrene (25.5%), 1,8-cineole (17.3%), γ-terpinene (10.7%), *p*-cymene (10.1%), terpinen-4-ol (7.5%), β-pinene (7.2%), α-thujene (4.9%)
Myoga (flower buds)	Eudesma-4 (14%), 11-diene (28.8%), β-elemene (26.5%), germacrene B (9.8%), β-eudesmol (4.0%), cycloundecatriene (4.0%), sabinene (3.5%)
Spearmint (leaves)	Carvone (80.0%)
Tea tree (leaves)	Terpinen-4-ol (39%)

[a] Constituents at levels of more than 3.0% are listed; [b] This constituent was detected only by GC/MS; [c] 6-Ethenyl-3-isopropyl-2-methoxy-6-methyl-5-isopropyl-cyclohex-3-ene; [d] 6-Ethenyl-3,6-dimethyl-5-iopropenyl-tetrahydrobenzofuran.

isopropyl-5-isopropenyl-6-methyl-2-methoxy-cyclohexane (a positional isomer of methyl-elemol), germacra-trien-6-one, furanosesquiterpene, and β-elemene, along with monoterpenes such as 1,8-cineole, camphene, and borneol. Kapurkachri oil mainly consisted of 1,8-cineole, β-eudesmol, elemol, and α-cadinol.

Gettou oil obtained in Okinawa contained mainly monoterpenes such as β-phellandrene, 1,8-cineole, γ-terpinene, *p*-cymene, and terpinen-4-ol. Myoga oil consisted of sesquiterpenes such as eudesma-4,11-diene, β-elemene, germacrene B, and β-eudesmol. Spearmint and tea tree oils used as reference oils contained mainly (-)-carvone and terpinen-4-ol, respectively.

Figure 1 illustrates the inhibition of filamentation by ginger oil, ethanol oleoresin, and major constituents (citral, [6]-gingerol, and [6]-shogaol) at 50 μg/mL. In contrast to the non-treated control with filamentous forms, ginger oil from fresh mature rhizomes (Figure 1A) provided almost complete inhibition of filamentation, with only the yeast form appearing. Oleoresin (Figure 1B) inhibited both filament and yeast forms at a rate of 54.6%. Citral and [6]-shogaol (Figures 1D and 1E) almost completely inhibited filamentation, and [6]-gingerol (Figure 1C) inhibited filamentation at a rate of 70.7%.

Table 4 shows the anti-*Candida* activity of five ginger oils, along with two ginger oleoresins and seven reference oils. Ginger oils had potent inhibitory activity against filamentous formation by *C. albicans*. As is apparent from the IC_{50} and IC_{90}, 5 ginger oils had 4~8 times more potent than 2 ginger oleoresins and other oils belonging to Zingiberaceae family as well as reference oils. The IC_{50} value of ethanol oleoresin was 50 μg/mL, those of CO_2 oleoresin, ginger lillylily, and kapurkachri oils was 100 μg/mL. The remaining plai, spearmint, and tea tree oils had an IC_{50} of 200 μg/mL. Gettou oil was weakest, with an IC_{50} of 400 μg/mL.

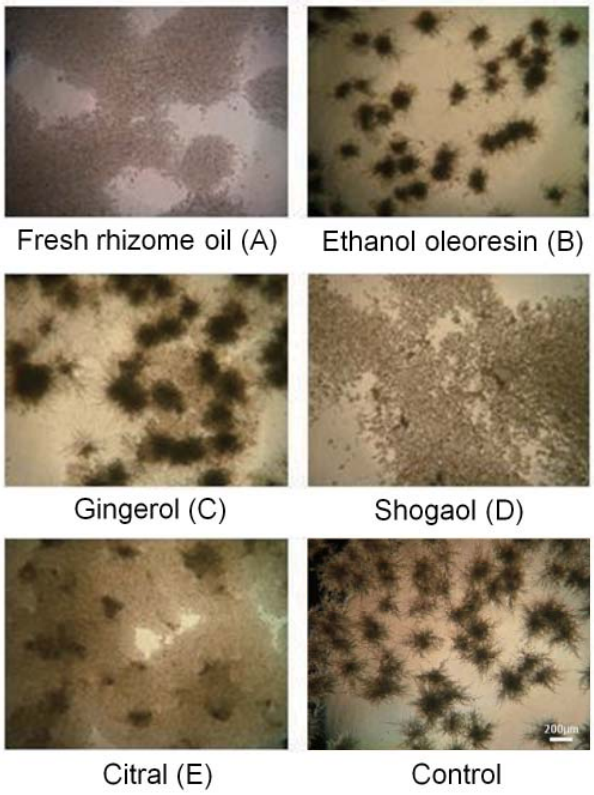

Figure 1. Inhibition of filamentation by fresh rhizome oil (A), ethanol oleoresin (B), [6]-gingerol (C), [6]-shogaol (D), and citral (E) at 50 μg/mL.

Table 4. Anti-*Candida* activity of ginger oils, oleoresins, and other essential oils belonging to the family Zingiberaceae and constituents

Agents	Inhibitory activity				
	Filament		Growth (RPMI)	Growth (YPG)	
	IC_{50} (μg/mL)	IC_{90} (μg/mL)	MIC (μg/mL)	Inhibition rate at 4,000 μg/mL (%)	IC_{50} (μg/mL)
Ginger oils					
Young	12.5	50	400	75.9%	2,000
Mature	12.5	50	400	98.0%	2,000
Seed	12.5	25	400	97.8%	1,000
Air-dried	12.5	25	200	97.5%	1,000
Steamed	12.5	50	nt	nt	nt
Ginger oleoresins					
CO_2 extract	100	200	> 800	65.9%	2,000
Ethanol extract	50	100	400	70.3%	2,000
Plai oil	200	400	1,600	56.2%	4,000
Ginger lily oil	100	200	> 3,200	39.9%	> 4,000
Kapurkachri oil	100	200	> 3,200	36.7%	> 4,000
Gettou oil	400	400	3,200	48.8%	4,000
Myoga oil	100	200	nt	nt	nt
Spearmint oil	200	400	1,600	43.9%	> 4,000
Tea tree oil	200	400	3,200	35.0%	> 4,000
[6]-Gingerol	50	100	> 100	nt	nt
[6]-Shogaol	6.25	25	100	nt	nt
β-Bisabolene	400	> 400	> 400	nt	nt
Citral	25	50	200	nt	nt

nt: not tested.

Table 5. DPPH radical scavenging activity of essential oils of Zingiberaceae plants, ginger oleoresins, [6]-gingerol, citral, and reference compounds

Oil, oleoresin, constituent	EC_{50}* (μg/mL)
Ginger oil (fresh mature rhizomes)	> 4,000
Plai oil	4,000
Ginger lily oil	4,000
Kapurkachri oil	> 4,000
Gettou oil	> 4,000
Ethanol oleoresin of ginger	62.5
CO_2 oleoresin of ginger	125
[6]-Gingerol	7.8
Citral	> 4,000
Eugenol	7.8
α-Tochopherol	62.5

* EC_{50}: effective concentration to decrease the initial DPPH concentration by 50%.

Ginger oils had more potent inhibition of *C. albicans* growth than did other oils. Ginger oils from air-dried rhizomes had an MIC of 200 μg/mL in RPMI-1640 medium. Three other ginger oils had an MIC of 400 μg/mL. Ethanol oleoresin had activity comparable to that of mature and young rhizomes, while CO_2 oleoresin and other oils were less able to inhibit growth compared to ginger oils. When the medium was changed from RPMI-1640 to YPD, the anti-*Candida* activity decreased considerably to an IC_{50} value of 1,000-2,000 μg/mL. Two oleoresins had comparable activity, but other oils, including the reference oils, had weaker activity.

Table 5 shows the DPPH radical scavenging activity of essential oils and oleoresins, expressed as EC_{50} values. Ethanol oleoresin from fresh ginger rhizomes exhibited potent activity as indicated by an EC_{50} of 62.5 μg/mL, comparable to that of α-tochopherol. CO_2 oleoresin had an EC_{50} of 125 μg/mL, but 5 essential oils including ginger, plai, ginger lily, kapurkachri, and gettou oils had very weak activity ≥ 4,000 μg/mL. [6]-Gingerol alone had an EC_{50} of 7.8 μg/mL, which was comparable to that of eugenol but more potent than that of α-tochopherol. Essentially, citral had no activity under the conditions tested.

4. Discussion

4.1. *Oil compositions*

In the growing stage, geranyl acetate is reportedly converted to geraniol and then to citral (*11*). This may be the reason for the high geranyl acetate content in young rhizomes. Terpinen-4-ol was major constituent of young hydrosol but was not detected in the young rhizome oil, suggesting that terpinen-4-ol was mostly transferred to the aqueous layer by steam distillation. Citral content increased more in seed rhizome oil than in fresh rhizomes, which agrees with the literature (*11*). The high citral content in the air-dried rhizomes may be due to the increased production of citral during the drying process. Citral content is reported to increase during storage at 15°C (*11*).

Oleoresins and ethyl acetate extracts had quite a low citral, geraniol, and geranyl acetate content. The low citral content in oleoresin in comparison to essential oil has been noted by other researchers (*12*). This suggests that citral and geraniol might be formed during steam distillation as a result of thermal decomposition. An increase in ar-curcumene to compensate for zingiberene in steam-heated rhizomes may be due to the thermal conversion of zingiberene (*13*). Lack of monoterpene hydrocarbon in ethanol oleoresin may be due, at least partly, to evaporation loss being accompanied by solvent distillation of ethanol and water.

As the current study determined, plai oil had a composition similar to that of oils found in Indonesia (*14*) and Bangladesh (*15*).

Ginger lily oil had a composition that differed vastly from that the composition reported in China, which included β-*trans*-ocimenone (28.05%) and linalool (18.5%) (*16*), suggesting a different chemo-type.

Kapurkachri oil had a composition similar to that reported by Bottini *et al*. (*17*). However, agarospirol, cadina-dienes, and cubenol were detected only in the oil studied here. Kapurkachri from southern India had a considerably different composition, according to Sabulal *et al*. (*18*).

Seasonal changes in the composition of gettou oil were studied in detail by Murakami *et al*. (*19*), and they reported that *p*-cymene, 1,8-cineole, and terpinen-4-ol were major constituents. These results agree with those of the current study. Qing *et al*. reported that oil from the flowers of myoga had a composition that included β-phellandrene, α-humulene, β-elemene, α-phellandrene, β-pinene, and β-caryophyllene (*20*). β-Elemene was found to be a common constituent, but other constituents were not found in the oil studied here.

4.2. *Anti-Candida activity*

Singh *et al*. reported that ginger oil had potent activity against *Fusarium moniliforme* but moderate or weak activity against other fungi and bacteria (*12*). They also stated that essential oil of ginger had more potent activity than did the corresponding oleoresin, which agrees with the current study.

Ginger oil is reported to have an MIC ranging from 1,600-3,200 μg/mL (average: 3,100 μg/mL) against *C. albicans* (*21*); in the current study, the oil had an MIC of 1,000-2,000 μg/mL in YPD medium but an MIC of 200-400 μg/mL in RPMI-1640 medium. The larger MIC in YPD medium was probably due to the faster rate of growth.

Citral was dominant in ginger oils except for that from steam-heated rhizomes. Since citral itself

exhibited potent activity as shown in Table 4, it may be responsible for the oils' inhibition of filamentation and growth of *C. albicans*. β-bisabolene, one of the major sesquiterpenes in ginger oil, and β-caryophyllene had weak activity. Although they were not tested, other sesquiterpenes in ginger oil such as zingiberene did not appear to have significant activity.

Two oleoresins did not contain citral, and their inhibitory activity may be due to the potent activity by [6]-gingerol itself, as shown in Table 4. Ethanol oleoresin had a higher level of activity than did CO_2 oleoresin, which may be attributed to higher [6]-gingerol content as shown in Table 2. The inhibition of *C. albicans* growth by ginger oil has previously been reported (*22*), but inhibition of filamentation by ginger oil, oleoresin, or gingerol has not been reported. The mechanisms of inhibition of growth and filamentation may differ (*23*), and the inhibition of filamentation cannot not be predicted based on inhibition of growth.

C. albicans displays dimorphism with yeast and filamentous, or hyphal, forms and its filamentous form causes candidiasis (*24*,*25*). Furthermore, the filamentous form contributes to biofilm formation at the infection site to disturb the action of antimicrobial agents (*26*). Since ginger oil inhibits filamentation as well as growth of the yeast form, it may have use as a mouthwash to prevent oral candidiasis.

However, ginger oil had no significant DPPH radical scavenging activity. Instead, ginger oleoresin, and especially ethanol oleoresin, exhibited scavenging activity comparable to α-tochopherol. Since [6]-gingerol is an active pungent principal of ginger and is reported to have anti-oxidative (*27*) and anti-inflammatory activities (*28*), gingerol-rich oleoresin may provide protection from skin disorders and offer antioxidant and health benefits as a food additive.

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