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Antifungal activity and chemical composition of ginger essential oil against ginseng pathogenic fungi

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Abstract

A large number of common herbs possess antimicrobial activity, because of their bioactive components, and some of them have become new potential anti-infective agents. In the present study, the antifungal activity of the essential oil from Ginger (*Zingiber officinale* Rosc.) was tested. The compositions of the oil was analyzed by GC/MS. Minimum inhibitory concentrations (MIC) against six pathogenic fungi causing ginseng root rot disease were determined for the essential oil. Ginger essential oil possessed significant antimicrobial effects against all phytopathogenic fungi tested. Only 0.3 % (v/v) concentration of ginger oil exhibited complete inhibition against *Alternaria panax*, *Botrytis cinerea*, *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, and *Sclerotinia nivalis*. The major constituents of ginger (*Zingiber officinale*) oil were citral (2, 6-octadienal, 3, 7-dimethyl) (76.94%), verbenyl ethyl ether (3.98 %), geranic acid (2.57%) and artemiseole (1.12%). The results of this investigation show evidence that the essential oil of ginger represent a potentially rich source for natural antimicrobials and may be useful as alternative anti-infectious agent to control ginseng root rot fungi.

Key words – ginseng root rot – natural antimicrobials – *Zingiber officinale*

Introduction

Ginseng plant (*Panax ginseng*) is a main agricultural product in South Korea. In recent years, Korea produced as much as 20 million pounds of dried ginseng roots per year from cultivated crops. Most of this was shipped to China, Japan, and other countries (Eo et al. 2014). Korean ginseng has a high susceptibility of both its roots and shoots to infection by various pathogens (Kang et al. 2007). Opportunistic fungi e.g. *Botrytis*, *Alternaria*, and *Fusarium* can infect ginseng roots and cause symptoms of discolored leaves, red perimeters. The roots gradually shrink underground until only a dark stub remains. Moreover, *Cylindrocarpon* often attacks older roots. The ginseng has low productivity compared to other plant crops, so these fungal infections greatly diminish the ginseng crop value which disrupts its purchase in markets (Davis et al. 2014). Therefore, to overcome these problems, most ginseng fields are treated by agricultural chemicals. Recently, both native and foreign ginseng farmers have begun to pay attention to the sustainable agriculture and clean technology, with a simultaneous increase in the use of natural antimicrobial compounds for organic and clean farming (Eo & Eom 2009, Kil et al. 2012). A large number of

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plants possess antimicrobial activity (Voravuthikunchai et al. 2004, Mothana & Lindequist 2005), and some active compounds of them have become possible source of new antimicrobial agents (Agunu et al. 2005, Buwa & Van Staden 2006). Essential oil, herbal extract, is well known for its antimicrobial activity (Friedman et al. 2002, Kalemba & Kunicka 2003). It is widely incorporated in food and medicine industry for this purpose. Among the various groups of plant products, essential oils are principally recommended as one of the most likely groups of natural products in the terms of safer antifungal agent's formulation (Varma & Dubey 2001). Mostly all of the essential oils were classified as generally recognized as safe (GRAS) and possess low risk for resistance evolution in pathogenic microorganisms (Cardile et al. 2009).

The demand for essential oils, as active ingredients, increased due to continued consumers' preference for minimally processed food products (Sadaka et al. 2013). In this regard, essential oils and more precisely bioactive molecules of them are being comprehensively analyzed for their insecticidal (Wu et al. 2016), antimicrobial (Burt 2004, Sökmen et al. 2004, Kordali et al. 2005), antifungal (Kordali et al. 2005), antiviral (Sökmen et al. 2004), and antioxidant attributes (Sökmen et al. 2004, Kordali et al. 2005). Generally, whole essential oils possess greater antifungal activity because of the synergistic effect with their active components; therefore, they are more promising for commercial application than single compounds (Tian et al. 2012). Essential oils are generally anti-infective natural substances and relatively less risky to humans, so they can be used for greenhouses pest management (Regnault-Roger et al. 2012). Pinto et al. (2009) stated that the antifungal azole drug mainly disrupts sterol biosynthetic pathways causing a decrease in ergosterol biosynthesis which inhibits fungal cell growth. Moreover, dill oil treatment resulted in an elevation of mitochondrial membrane potential. Dill oil also decreased ATPase and dehydrogenase in *A. flavus* cells (Tian et al. 2012). In this study, the chemistry and antifungal activity of ginger essential oil against a range of ginseng-infecting fungi are scrutinized by giving special emphasis to its potential management for ginseng root rot disease.

Materials & methods

Sampling and fungal strains

The fungi used for the bioassay test were pathogenic isolates collected from the infected ginseng roots in the laboratory of Soil Microbiology, Department of Biological Environment, Kangwon National University, Korea. Infected plants were uprooted, placed into zipper bags, and transported to the laboratory. Fungal isolates were cultivated on potato dextrose agar (PDA) using a tiny isolation needle. Different species were isolated and purified based on their distinctive colonies shapes using dilution plate technique on Czapek's solution agar containing saccharose 20 g; sodium nitrate 3 g; dipotassium phosphate 1 g; magnesium sulfate 0.5 g; potassium chloride 0.5 g; ferrous sulfate 0.01 g; Agar 15 g. Stock cultures of fungi were maintained on 2% malt extract-agar (MEA) plates grown at 24 °C and stored at 4 °C.

Fungi identification

The fungal strains isolated from infected *P. ginseng* samples were identified by the microscopic examination and the culture features according to Domsch et al. (1980) and Moubasher (1993). Six fungal species were selected for this study (*Alternaria panax*, *Botrytis cinerea*, *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, and *Sclerotinia nivalis*). Their identification were confirmed as pathogenic strains by the Korean Agricultural Cultural Collection (KACC), Jeonju, Korea.

Agar dilution test

The ginger essential oil (EO) used in this experiment was 100% pure natural product purchased from the manufacturer (Sydney Essential Oil Co. Pty. Ltd., Australia). Antifungal assays were achieved in triplicates and the results were averaged. In this technique, different dilutions of an EO was made in potato dextrose agar (PDA) medium, and then mycelial discs of actively

growing 5 day old pathogens were inoculated on the PDA using a cork borer with 6 mm diameter, as recommended by Jiang (2011). Agar dilution is a proper method to create a saturated moistened atmosphere to adjust volatility (Pauli & Schilcher 2010). PDA plates without EO served as control. The plates were incubated at 27 °C for 10 days. Observations on the antimicrobial activities of EOs on the phytopathogenic fungi were recorded after 10 days and inhibition percentage was calculated using the formula of Messgo-Moumene et al. (2015):

$$\text{Inhibition percentage (\%)} = C_1\text{-}C_2/C_1 \times 100$$

Where, C_1 is the colony area of untreated pathogenic fungus in the control, and C_2 is the colony area of pathogenic fungus in dual culture.

GC-MS analysis

The essential oil extract was subjected to gas chromatography–mass spectroscopy (GC-MS) analysis for identification of the oil components in the central laboratory, Kangwon National University, Gangwon province. This was carried out using a GC (Agilent 7890A)/ MSD (GEOL JP/JMS-Q1050GC). Identification of the chemical constituents of the essential oil was accomplished by following their retention indices and mass spectra with those present in Wiley 275 library (Okoh et al. 2010). The quantities of compounds were calculated by integrating the peaks area of the spectrogram. A needle with the sample materials (essential oil tested) was introduced directly into the inlets of the gas chromatograph. The initial temperature 50 °C, maximum temperature 250 °C, equilibration time 3 min, ramp 5 °C/min, ultimate temperature 250 °C; inlet: splitless, initial temperature 250 °C, pressure 8.27 psi, purge flow 1mL/min, He, purge period 0.20 min, gas type helium; the column capillary, 30×0.32 mm i.d., 0.5㎛ film thickness first flow 0.7 ml/min, velocity average 32 cm/s; MS: El method at 75 eV.

Statistical analysis

Statistical analysis was performed on the all data with SAS (SAS Institute 2011) using Tukey's test, version 11.0, to determine the significant differences between mean values and to compare the means ($P > 0.05$).

Results

Antifungal assay of ginger oil

All the tested phytopathogenic fungi of ginseng root rot were found to be sensitive to ginger essential oil. Ginger oil at 0.05% v/v exhibited 33.3% inhibition to *Alternaria panax*, 16.5% inhibition to *Cylindrocarpon destructans*, 12.3% inhibition to *Fusarium oxysporum*, 11.6% inhibition to *Sclerotinia sclerotiorum*, 8.1% inhibition to *Sclerotinia nivalis*, and 5.6% inhibition to *Botrytis cinerea* (Fig. 1). Ginger oil at 0.1% (v/v) exhibited complete inhibition 100% against the pathogenic fungi *Cylindrocarpon destructans*, *Sclerotinia sclerotiorum* and *S. nivalis*, weaker toward *Alternaria. panax* (90.6%), *Fusarium oxysporum* (56.7%), and *Botrytis cinerea* (17.5%) (Table 1). Thus the minimum inhibitory concentration (MIC) value of ginger oil against *Cylindrocarpon destructans*, *Sclerotinia sclerotiorum* and *Sclerotinia nivalis* was (0.1% v/v). However, 0.3% (v/v) was MIC value of ginger oil to suppress all the investigated fungi of ginseng root rot.

Chemical composition of ginger oil

The various chemical components identified in the essential oils of *Zingiber officinale* are shown in Table 2, oxygenated monoterpenes were the dominant constituents in the *Z. officinale* oil (10 compounds, 92.12%) of which citral (2, 6-octadienal, 3, 7-dimethyl) (76.94%) was the major compound followed by verbenyl ethyl ether (3.98 %) with lower amount of geranic acid (2.57%) and artemiseole (1.12%). A lower percentage (7.6%) of aliphatic hydrocarbons (10 compounds)

was detected with oxirane (1.92%), 2, 7-Dimethyl-2, 7-octanediol (1.67%) and 2-Hexenoic acid-5-oxo, 3, 4, 4-trimethyl (0.31%). The essential oil from ginger also contained alcohols (5.58%) and ketones (3.83%) in low quantity. The sesquiterpene hydrocarbons represented 82.47% of the total oil. Twelve sesquiterpenes skeletons types (limonene, pinene, camphor, linalool, citral, piperitone type, geranial, carveol, nerol, neric acid, geranic acid, artemiseole) were identified from the ginger essential oil.

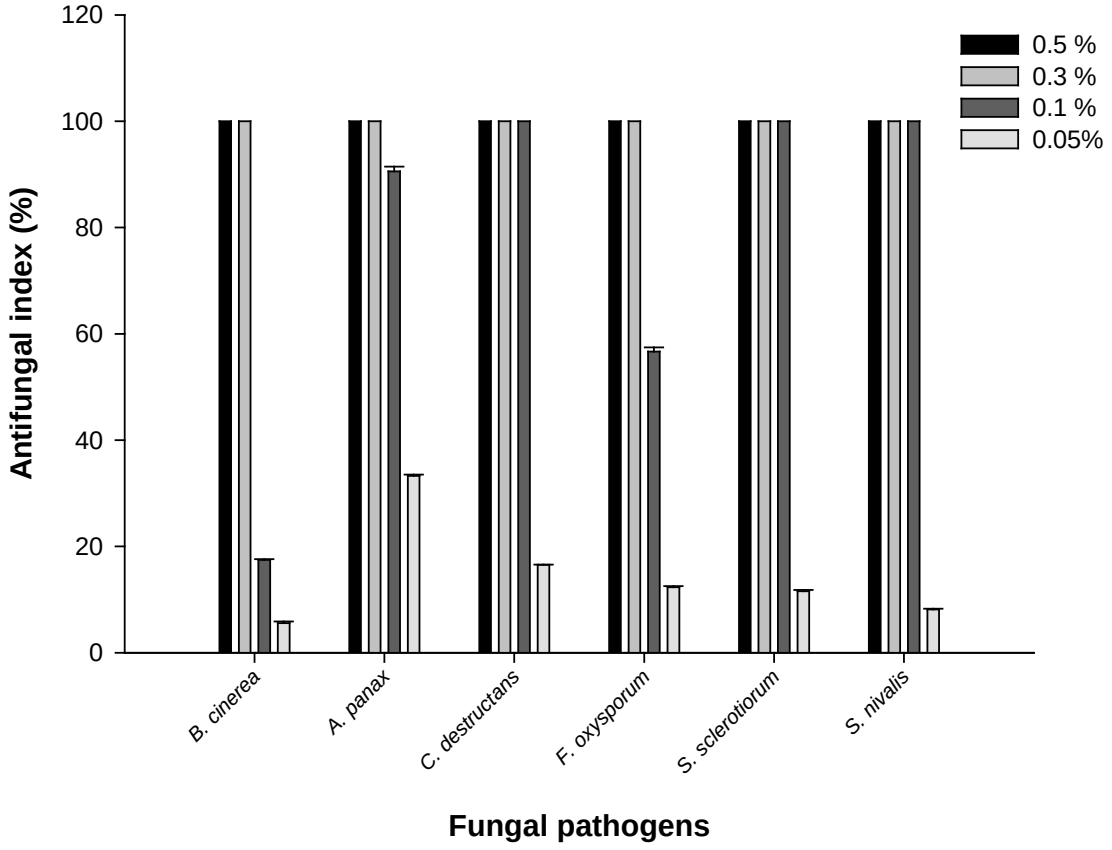

Fig. 1 – Antifungal activity of the essential oil of ginger against ginseng root rot fungi. *Botrytis cinerea*; *Alternaria panax*; *Cylindrocarpon destructans*; *Fusarium oxysporum*; *Sclerotinia sclerotiorum*; *Sclerotinia nivalis*. The phytopathogenic fungi (agar dilution tests) were examined after 10 days and the inhibition percentages were calculated.

Table 1 Antifungal indices of ginger essential oil

Pathogenic fungi	Ginger oil concentration (%)			
	0.05	0.1	0.3	0.5
Botrytis cinerea	5.6 ± 0.31f	17.5 ± 0.15d	100 ± 0.0a	100 ± 0.0a
Alternaria panax	33.3 ± 0.25a	90.6 ± 0.90b	100 ± 0.0a	100 ± 0.0a
Cylindrocarpon destructans	16.5 ± 0.06b	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
Fusarium oxysporum	12.3 ± 0.21c	56.7 ± 0.76c	100 ± 0.0a	100 ± 0.0a
Sclerotinia sclerotiorum	11.6 ± 0.25d	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
Sclerotinia nivalis	8.1 ± 0.15e	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a

Different letters indicate significant difference (P<0.05) between control and treatments.

Table 2 Chemical compositions of ginger essential oil

Peak No.	Compound	RT (min)	Concentration (%)
1	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	06:28	0.94
2	2-Buten-1-ol, 3-methyl-	09:52	0.19
3	Allyldimethyl(prop-1-ynyl)silane	10:17	0.29
4	1-Butene, 2,3-dimethyl-	10:28	0.11
5	3-Octanol, acetate	11:20	0.92
6	2H-Pyran, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-	11:29	0.28
7	6-Bromomethyl-5-methyl-bicyclo[3.1.0]hexan-2-one	12:06	0.08
8	3-Oxabicyclo[3.3.0]octan-2-one, 7-methylene-4,4-dimethyl-	12:40	0.19
9	Ethanone, 1-(1-methyl-2-cyclopenten-1-yl)-	12:46	0.04
10	4,5-Heptadien-2-one, 3,3,6-trimethyl-	13:22	0.21
11	1,4-Hexadiene, 3,3,5-trimethyl-	14:40	0.09
12	1,6-Octadien-3-ol, 3,7-dimethyl-	15:23	0.65
13	Artemiseole	15:31	1.12
14	Oxirane, (1,1-dimethylbutyl)-	16:24	1.92
15	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	17:10	0.52
16	Verbenyl ethyl ether	17:47	3.98
17	Isoborneol	18:05	1.18
18	2,6-Octadienal, 3,7-dimethyl-	18:52	76.94
19	2-Isopropylidene-5-methylhex-4-enal	18:57	0.95
20	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	19:47	1.13
21	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	20:14	0.98
22	2,7-Dimethyl-2,7-octanediol	25:12	1.67
23	trans-2-[2'-(2''-Methyl-1''-propenyl)cyclopropyl]propan-2-ol	26:34	1.89
24	2-Hexenoic acid, 3,4,4-trimethyl-5-oxo-	26:43	0.31
25	Neric acid	30:44	0.88
26	Geranic acid	31:34	2.57

Discussion

Ginger, botanically known as *Zingiber officinale* (family: Zingiberaceae), is one of the most frequently used spices worldwide with medicinal significance. Ginger has been recommended for a numerous of medical conditions and is claimed to have carminative, spasmolytic, expectorant, appetite stimulant, anti-inflammatory, and digestive effects (Tracy et al. 2007). Ginger oil has been estimated to possess antimicrobial effects (Preedy 2016). In our investigation, all phytopathogenic fungi of ginseng root rot were found to be sensitive to ginger essential oil. Natta et al. (2008) demonstrated that the essential oil of ginger obtained by hydrodistillation involves high antibacterial effects on food pathogens (*Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*), with MIC of 6.25 μg/mL to inhibit *Listeria monocytogenes* and *Botrytis. cereus*. Subsequent studies have demonstrated that the oil extracted from the rhizome and leaves was moderately active against the Gram-positive bacteria, *S. aureus*, *Bacillus licheniformis*, and *Bacillus spizizenii*, and the Gram-negative bacteria *Pseudomonas stutzeri*, *Escherichia coli*, and *Klebsiella pneumoniae* (Sivosathy et al. 2011). Our investigation revealed that ginger oil at 0.05% v/v exhibited 33.3% inhibition to *Alternaria panax*, 16.5% inhibition to *Cylindrocarpon destructans*, 12.3% inhibition to *Fusarium oxysporum*, 11.6% inhibition to *Sclerotinia sclerotiorum*, 8.1% inhibition to *Sclerotinia nivalis*, and 5.6% inhibition to *Botrytis cinerea* (Fig.

1). Ginger oil at 0.1% (v/v) exhibited complete inhibition 100% against the pathogenic fungi *Cylindrocarpon destructans, Sclerotinia sclerotiorum* and *S. nivalis*, weaker towards *Alternaria panax* (90.6%), *Fusarium oxysporum* (56.7%), and *Botrytis cinerea* (17.5%) (Table 1). Thus the minimum inhibitory concentration (MIC) value of ginger oil against *Cylindrocarpon destructans*, *Sclerotinia sclerotiorum*; *Sclerotinia nivalis*.was (0.1% v/v). However, 0.3% (v/v) was MIC value of ginger oil to suppress all the investigated fungi of ginseng root rot. The different effect of EO on the fungal species may be due their effect on biosynthesis and organelles rather than only cell wall. Some previous studies have demonstrated that natural and synthetic antifungal agents can cause a considerable lessening in the quantity of ergosterol which is the major sterol component in the fungal cell membrane. Ergosterol is also responsible for maintaining cells function and integrity (Tian et al. 2012). Fig. 2 illustrates the antifungal activity of the gingers' essential oil toward the ginseng root rot pathogenic fungi. Sasidharan & Menon (2010) have revealed that the fresh oil of ginger was effective inducing the antimicrobial effects on *Aspergillus niger*, *Candida*, and *P. aeruginosa*, and less effective against *Saccharomyces cerevisiae*, and inactive against *B. subtilis*, *Penicillium* spp., and *Trichoderma* spp. However, the dry ginger oil was more active against *P. aeruginosa*, *B. subtilis*, *S. cerevisiae*, *A. niger*, and *Penicillium* spp. Fresh ginger oil had MIC value of <1 μg/mL against *A. niger* and *Candida albicans*, and dry ginger oil had an MIC value of less than 1 μg/mL against *Pseudomonas aeruginosa*, *Penicillium* spp. and *Candida albicans*. Fresh ginger is more plentiful in oxygenated compounds, and the observed difference in the antimicrobial effects is imputed to this factor (Sasidharan & Menon 2010).The various chemical components identified in the essential oils of *Zingiber officinale* are shown in Table 2, oxygenated monoterpenes were the dominant constituents in the *Z. officinale* oil (10 compounds, 92.12%) of which citral (2, 6-octadienal, 3, 7-dimethyl) (76.94%) was the major compound followed by verbenyl ethyl ether (3.98 %) with lower amount of geranic acid (2.57%) and artemiseole (1.12%). A lower percentage (7.6%) of aliphatic hydrocarbons (10 compounds) was detected with oxirane (1.92%), 2, 7-Dimethyl-2, 7-octanediol (1.67%) and 2-Hexenoic acid-5-oxo, 3, 4, 4-trimethyl (0.31%). The essential oil from ginger also contained alcohols (5.58%) and ketones (3.83%) in low quantity. The sesquiterpene hydrocarbons represented 82.47% of the total oil. Twelve sesquiterpenes skeletons types (limonene, pinene, camphor, linalool, citral, piperitone type, geranial, carveol, nerol, neric acid, geranic acid, artemiseole) were identified from the ginger essential oil (Fig. 3). The principal chemical constituents were found to be 2, 6- octadienal, 3, 7-dimethyl (citral) (76.94.1%), verbenyl (3.98%), geranic acid (2.58%), and isoborneol (1.18%). Sa-Nguanpuag et al. (2011) found that the major constituents of ginger oil were camphene, 1, 8-cineol, and β-phellandrene. The differences in chemical composition of *Z. officinale* essential oil may partly refer to the difference in the extraction techniques, geographical sources and maturity stages of *Z. officinale*. Kiran et al. (2013) studied the influence of cultivar on essential oil yield and major chemical composition, and found that, among all studied cultivars, ginger of Assam Tinsukia had the highest citral content (23.66%) and Meghalaya mahima ginger had the highest zingiberene content (29.89%). Nevertheless, compounds of *Z. officinale* essential oil collectively exhibited growth inhibition effect on all phytopathogenic fungal strains tested in present study. The composition of raw ginger oil has shown that the content of citral is higher compared to the oil from dried plant material. The Cochin ginger variety has shown a yield of 1.5–2.2% of an oil rich in citral. The other monoterpenes of low boiling point like neral, cineole, borneol, geraniol, geranial, and α-pinene are less abundant and present in various proportions in dried ginger varieties. The chemical composition of fresh ginger essential oil shows that it contains more oxygenated compounds (29%) compared to dry ginger oil (14%) (Sasidharan & Menon 2010). The higher content of oxygenated compounds, such as geranial, makes fresh ginger oil more effective than dry ginger oil (Preedy 2016). However, the dry ginger oil contains more hydrocarbon compounds compared to fresh ginger oil (Preedy 2016). Fresh ginger is more plentiful in oxygenated compounds, and the observed difference in the antimicrobial effects is imputed to this factor (Sasidharan & Menon 2010). Fabra et al. (2008) included that essential oil of ginger is another alternative for improving the sodium caseinate-based edible packaging. Our results indicate

the importance of essential oils of common plants in the preservation and protection of ginseng crop from fungal pathogens.

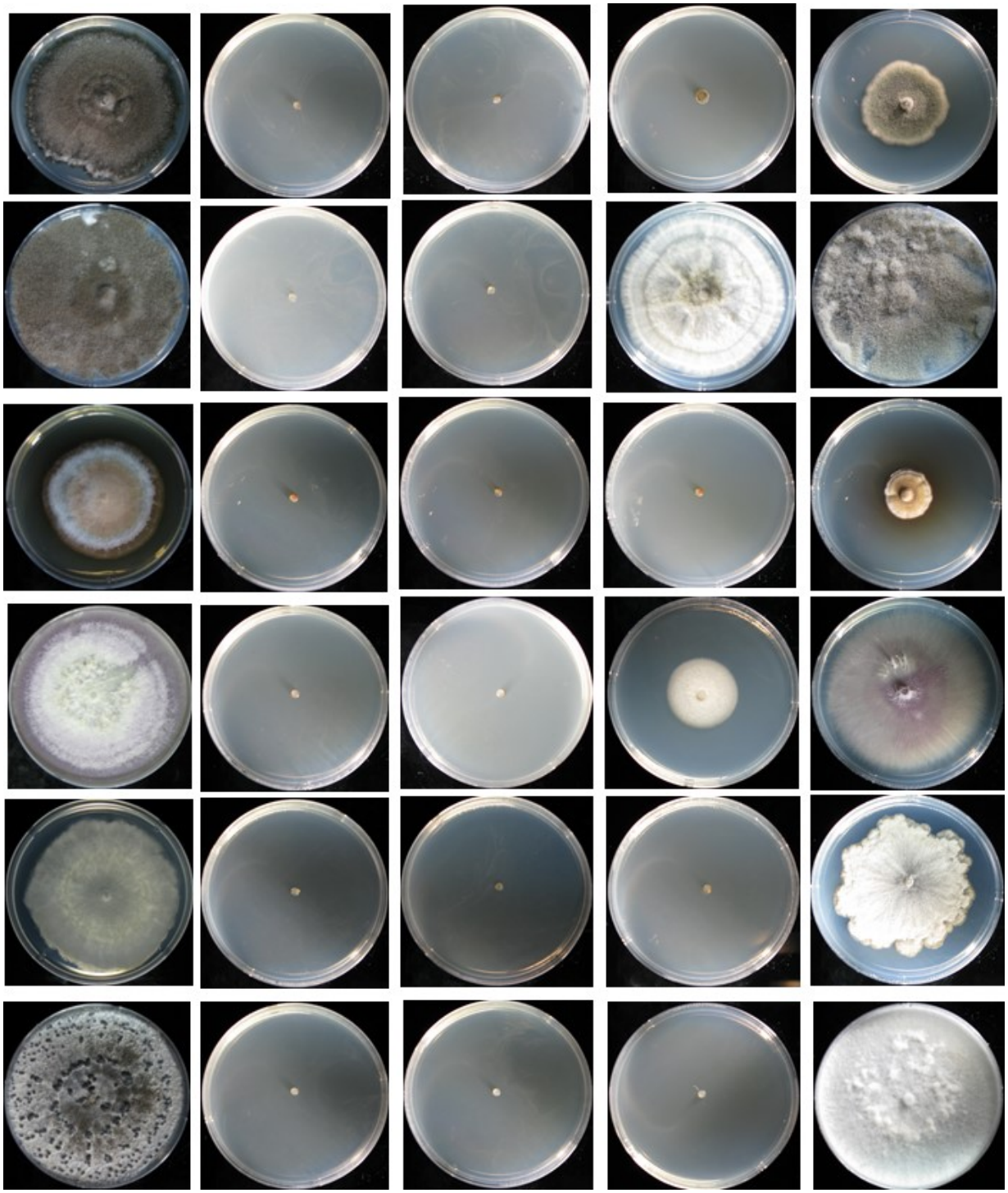

Fig. 2 – Antifungal activity of the gingers' essential oil (left to right) Control, 0.5, 0.3, 0.1, and 0.05 % against ginseng root rot fungi (up to down) *Botrytis cinerea*; *Alternaria panax*; *Cylindrocarpon destructans*; *Fusarium oxysporum*; *Sclerotinia sclerotiorum*; *Sclerotinia nivalis*. on agar dilution test, phytopathogenic fungi were examined after 10 days and the inhibition percentage was calculated according to the formula of Messgo-Moumene et al. (2015).

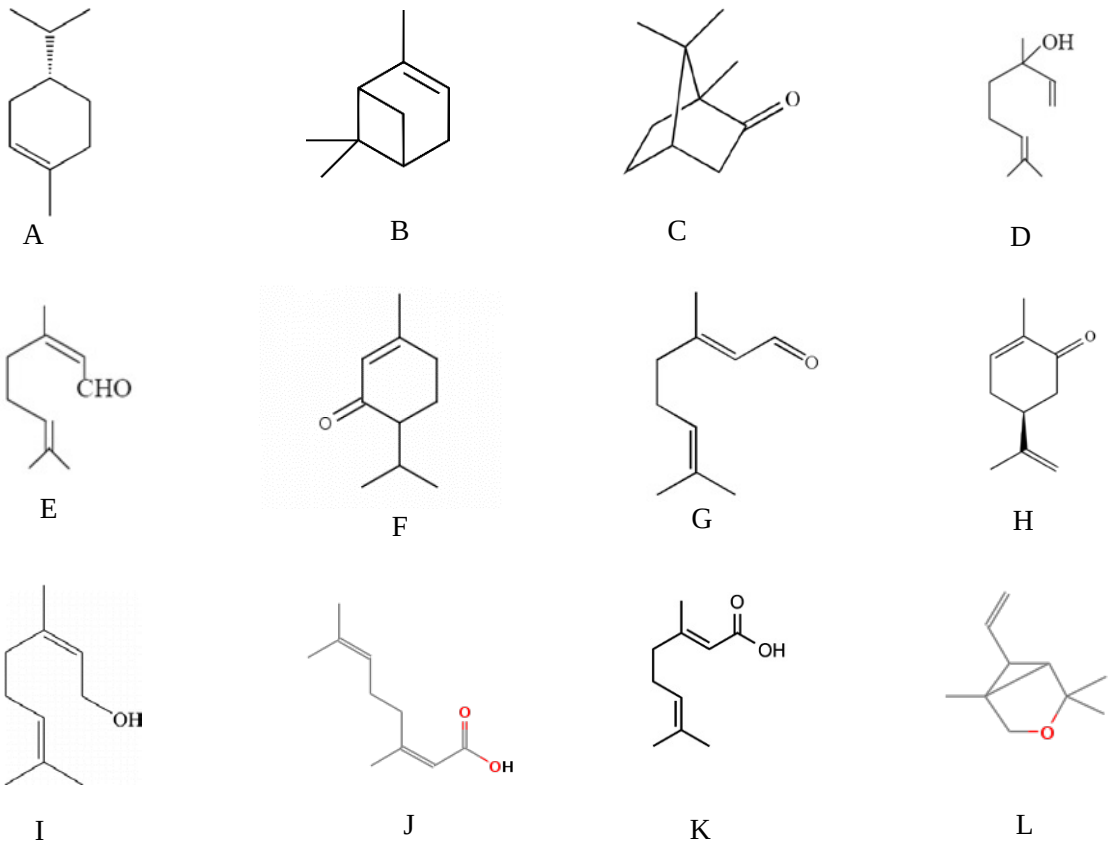

Fig. 3 – Main sesquiterpenes skeletons of ginger essential oil. A limonene type. B pinene type. C camphor type. D linalool type. E citral type. F piperitone type. G geranial type. H carveol type. I nerol type. J neric acid type. K geranic acid type. L artemiseole type.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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