academicJournals

Vol. 16(52), pp. 2349-2354, 27 December, 2017
DOI: 10.5897/AJB2017.16209
Article Number: 0149EE355255
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Chemical composition of neem and lavender essential oils and their antifungal activity against pathogenic fungi causing ginseng root rot

Khalid Abdallah Hussein[1,2] and Jin Ho Joo[1,2*]

[1]Botany and Microbiology Department, Faculty of Science, Assiut University, 71516, Assiut, Egypt.
[2]Soil Biochemistry Laboratory, Department of Biological Environment, Kangwon National University, Republic of Korea.

Received 17 August, 2017; Accepted 20 December, 2017

The aim of this study was to assess *in vitro*, the antifungal activity and characterize the chemical constituents of the essential oils of *Azadirachta indica* (neem) and *Lavandula angustifolia* (lavender). The essential oils of *A. indica* and *L. angustifolia* plants were tested against several isolates of pathogenic fungal genera causing root rot disease of ginseng. Agar plate assay indicated that lavender oil at 10% exhibited the highest inhibition index of (86.0±0.7%) against *Sclerotinia nivalis* mycelial growth. Neem and lavender oils at 5% v/v showed inhibition index against *Alternaria panax* (72.9±2.1 and 45.0±1%, respectively). Lavender oil at 5% (v/v) inhibited growth of *S. nivalis* (83.1±0.2%) and *Cylindrocarpon destructans* (49.2±1%). The gas chromatography-mass spectrometry (GC-MS) analysis showed that the major constituents of neem oil were fatty acids (94.8%). However, sesquiterpenes were the dominant constituents of the lavender oil (57.6%). The antifungal indices demonstrated in this study are a clear evidence of the potentiality of neem and lavender essential oils to control plant diseases caused by phytopathogenic fungi.

Key words: Ginseng root rot, fungi, essential oils.

INTRODUCTION

Korean ginseng is well known as a very important economic herb plant cultivated and used in Asia. The medicinal value of Korean ginseng (*Panax ginseng*) has been discovered for over a thousand years (Baeg and So, 2013; Shishtar et al., 2014). Mainly, the pharmacological active compounds in ginseng are in the roots. Long cultivation time maximizes the concentrations of these vital compounds in root. Therefore, in Korea, ginseng plants are commonly cultivated for 4 or 6 years, usually in shady zones. However, continual cultivation in the same soil for a long duration leads to a decline in the fertility and physicochemical properties of the soil, and provide favorable conditions for infection by fungal soil-borne pathogens which cause severe damage in yield due to soil borne pathogens, such as *Fusarium* spp. and *Cylindrocarpon* spp., that can exist as highly virulent strains on ginseng and coniferous hosts (Seifert et al., 2013). *Cylindrocarpon* attacks only root, usually older

*Corresponding author. E-mail: jhjoo@kangwon.ac.kr. Tel: +82-33-250-6448. Fax: +82-33-241-6640.

ones (Davis and Persons, 2014). Chemical application of pesticides is a handy approach to control disease in ginseng plantations. However, the accumulation of undesirable pesticide residues in the surrounding soil and even in ginseng roots has become a hazardous environmental problem.

Currently, essential oils are considered as broad-spectrum pesticides, organic pesticides, and low-risk pesticides whereas high-risk pesticides cannot be conveniently applied such as in schools, greenhouses and homes (Lang and Buchbauer, 2012). Essential oils are volatile aromatic liquids obtained from plant origin, including flowers, leaves, fruits, seeds, bark, peel, wood, roots and whole plants (Hyldgaard et al., 2012). The ancient Egyptians utilized essential oils in perfumery and in the embalming and preparing bodies for burial through mummification. Many essential oils have antioxidant and antimicrobial properties (Dandlen et al., 2011; Lang and Buchbauer, 2012), but their use as food preservatives requires more knowledge of their properties, including their antimicrobial potency, the specific mode of action, and the sensitivity of the target microorganisms (Hyldgaard et al., 2012). Antifungal activities of different essential oils have been evaluated (Tabassum and Vidyasagar, 2013; Kamal et al., 2012). Tabassum and Vidyasagar (2013) classified and characterized antifungal essential oils on the basis of the family of origin plant. Basil oil was effective against the fungi, *Penicillium aurantiogriseum*, *Penicillium glabrum*, *Penicillium chrysogenum* and *Penicillium brevicompactum* (Kocić-Tanackov et al., 2012). Sweet basil oil at 0.6% v/v showed 100% inhibition of mycelia growth against the rice pathogenic fungi, *Pyricularia arisea* and *Fusarium moniliforme* and 50% inhibition against *Fusarium proliferratum*, but was not effective against *Rhizoctonia solani*. Basil oil at 0.8% v/v inhibited spore germination of *F. monoliforme* (90%) and *Alternaria brassicicola* (100%) (Piyo et al., 2009). The aims of the present study were to estimate the antimicrobial potential of neem and lavender essential oils on the growth of pathogenic fungal strains causing ginseng root rot and to characterize the oils chemical composition.

MATERIALS AND METHODS

Sampling and fungal strains

The fungi used for the bioassay test were collected from the infected ginseng roots in the farm of Soil Microbiology Laboratory, 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 (g/L) 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 27°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 was confirmed as pathogenic strains by the Korean Agricultural Cultural Collection (KACC), Jeonju, Korea.

Agar dilution test

The experiment was conducted using 100% pure essential oil (EOs) of neem (*Azadirachta indica*) belonging to Meliaceae family and lavender belonging to Lamiacea family (*Lavandula angustifolia*) which were purchased from the manufacturer (Sydney Essential Oil Co. Pty. Ltd., Australia). Antifungal assays were conducted in three replicates and the average of the data was calculated. EOs were amended in PDA to make 5 and 10% concentration in Petri plates, respectively. Solidified agar plates were inoculated at the center with mycelial disc (6 mm) diameter of the pathogen and incubated at 27°C for 10 days. Plates without EOs served as control. The agar dilution test, a concentration gradient of the tested EOs, was used to evaluate the antifungal activity of tested substance (Lang and Buchbauer, 2012). The antimicrobial activities of EOs on the phytopathogenic fungi were examined after 10 days and the inhibition percentage was calculated according to the formula of Messgo-Moumene et al. (2015):

$$\text{Inhibition percentage (\%)} = (A_1-A_2)/A_1 \times 100$$

Where, A_1 is the colony area of untreated pathogenic fungus in the control and A_2 is the area of pathogenic fungus colony in dual culture.

Essential oil analysis

The essential oils composition was detected using GC-MS on a GC Agilent -7890A/MS GEOL JP/JMS-Q1050GC (GC/MS system) apparatus equipped with a DB-WAX column (30 × 0.32 mm, 0.5 µm film thickness). Helium was adopted as the carrier gas at 1.0 mL/min flow rate; column pulse pressure was 48.7 kPa; linear velocity was 36.0 cm/s; at a flow rate of 50 mL/min; the carrier flow rate was 24 mL/min; injector temperature was 250°C; detector temperature was 250°C; column temperature at 40°C (3 min) to 150°C (3 min) at 5°C/min, then 155 to 250°C at 10°C/min for 10 min. In the GC-MS detection, an electron ionization system was adopted with ionization energy of 75 eV. Samples were diluted 1/100 (v/v) in ethanol and 1.0 µL were injected in the splitless mode (Adams, 1995). The compounds were identified by matching their fragmentation patterns detected in the mass spectra with the patterns present in the NIST 98 mass spectrometry library (Central Lab., Kangwon National University, Korea). Quantification of the constituents was based on the peak area percentage of each constituent in relation to the total area of the ideal peaks in the chromatogram.

Statistical analysis

The statistical analysis was performed on all the data with SAS Institute (2011**)** using Tukey's test, version 11.0, to compare the means ($P > 0.05$).

Table 1. Antifungal indices of neem and lavender essential oils.

Species	Neem oil (%)		Lavander oil (%)	
	5	10	5	10
Alternaria panax	72.9 ± 2.1[a]	77.6 ± 0.9[a]	45 ± 1[c]	52.8 ± 0.7[c]
Sclerotinia sclerotiorum	52.9 ± 2.3[b]	69.7 ± 0.8[b]	5.6 ± 0.2[f]	11.5 ± 0.4[f]
Sclerotinia nivalis	50.3 ± 1.5[b]	66.4 ± 0.3[c]	83.1 ± 0.2[a]	86 ± 0.7[a]
Cylindrocarpon destructans	42.5 ± 1.7[c]	52.6 ± 0.6[d]	49.2 ± 1[b]	66 ± 0.8[b]
Botrytis cinerea	30.5 ± 0.9[d]	49.9 ± 0.6[e]	11.1 ± 0.1[e]	17.1 ± 0.5[e]
Fusarium oxysporum	24.6 ± 0.6[e]	31.6 ± 0.8[f]	14.8 ± 0.3[d]	31.1 ± 0.2[d]

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

RESULTS AND DISCUSSION

At least five species of fungi have been involved in some form of ginseng root rot: *Alternaria*, *Botrytis*, *Cylindrocarpon*, *Fusarium* and *Sclerotinia* (Davis and Persons, 2014; Schnitzler et al., 2011; Silva et al., 2011; Oussalah et al., 2007). Therefore, to overcome this problem, most ginseng fields are treated with synthetic agricultural chemicals (Tawaha et al., 2007; Moreira et al., 2005). However, there is a strong anxiety about the safety aspects of chemical pesticides, since they are considered as carcinogenic and responsible for many diseases, as well as residual toxicity (Peng et al., 2005).

Antifungal assay of essential oil

Six representative strains of ginseng root rot fungi were selected to test the antifungal activity of neem and lavender essential oils. The antifungal indices demonstrated in Table 1 are a clear evidence of the excellent antifungal activities of the tested oils. The antimicrobial activity obtained from neem oil was relatively higher than lavender oil. Neem oil showed the highest antifungal activity against *Alternaria panax* (77.6±0.6%), followed by *S. sclerotiorum* (69.7±0.8%). Moderate antifungal activity was recorded against *S. nivalis* (66.4±1.5%), *C. destructans* (52.6±1.7%) and *B. cinerea* (49.9±0.6%). *F. oxysporum* was the least sensitive fungus (31.6±0.8%) at 10% v/v. Lavender oil showed distinctive antifungal activity against all the tested fungi. The highest index of inhibition (86±0.7%) and the lowest (11.5±0.4%) were recorded against *S. nivalis* and *S. sclerotiorum*, respectively. Moderate antifungal activity was recorded against *F. oxysporum* (31.1±0.2%) and *B. cinerea* (17.1±0.5%). *C. destructans* showed inhibition index of 66±0.8% at dose of 10% v/v (Figures 1 and 2). Kamal et al. (2012) studied the antifungal activity of the ajwain seeds volatile oil. The volatile components of ajwain seeds inhibited 70 to 90% growth of 10 fungi *Acrophialophora fusispora*, *Alternaria grisea*, *Alternaria tenuissima*, *Curvularia lunata*, *Drechslera tetramera, F. chlamydosporum*, *F. poae*, *Myrothecium roridum*, *Papulaspora* sp. and *Rhizoctonia solani*. The essential oil of *Chamaecyparis formosensis* wood possessed antifungal activity, with an antifungal index of 88.2 and 67.3% for the wood decay fungi, *Laetiporus sulphureus* and *Trametes versicolor* at a dose of 50 mg ml^{-1}, respectively (Wang et al., 2005). Leaves extracts of neem which are inexpensive and environmentally safe, are promising for protection of economic plants against fungal infection leading to improvement of the crop regarding yield and productivity (Mondall et al., 2009). The data revealed that fatty acids (5 compounds, 94.02%) are the main class in the neem oil. The gas chromatographic analysis of neem oil showed the presence of hexadecanoic acid with the highest percentage (78.25%), followed by tetradecanoic acid (7.24%), oleic acid (3.64%), octadecenoic acid (3.5%), and linoleic acid (1.39%) (Table 2). Figure 3 shows the main fatty acids skeletons of essential oil from neem. Many studies have reported that majority of the fatty acid content of neem (*A. indica*) extract ranges between 25 and 61.9% (Djenontin et al., 2012). Sandanasamy et al. (2013) showed the presence of nine fatty acids, including four major ones. These are oleic acid (41.91±0.69%), followed by linoleic acid (19.59±0.44%), stearic acid (18.71±0.46%) and palmitic acid (15.59±0.27%). The differences in the fatty acids percentage and their composition may be attributed to the extraction method, plant species and their cultivation conditions (Atabani et al., 2013). Among them, 1,6-octadien-3-ol, 3,7-dimethyl- (41.74%) and silane, triethylfluoro (36.7%) were the major components in the lavender essential oil. Table 3 shows the constituents of lavender essential oil. Oxygenated monoterpenes (4 compounds, 57.62%) are the main class in the lavender oil, of which 1,6-octadien-3-ol,3,7-dimethyl (41.74%) was the major compound followed by bicyclo [1.2.2] heptan-2-one, 1,7,7-trimethyl (6.91%) with lower amounts of eucalyptol (5.99%) and 3-cyclohexene-1-methanol, a,a4-trimethyl (2.99%). Figure 4 shows the main sesquiterpenes skeletons of these compounds, a lower percentage (42.38%) of aliphatic hydrocarbons (2 compounds), silane, triethylfluoro (36.71%) and methanesulfonyl chloride (5.67%). However, Hui et al. (2010) showed that dimethyl vinyl hexenylbutyrate was

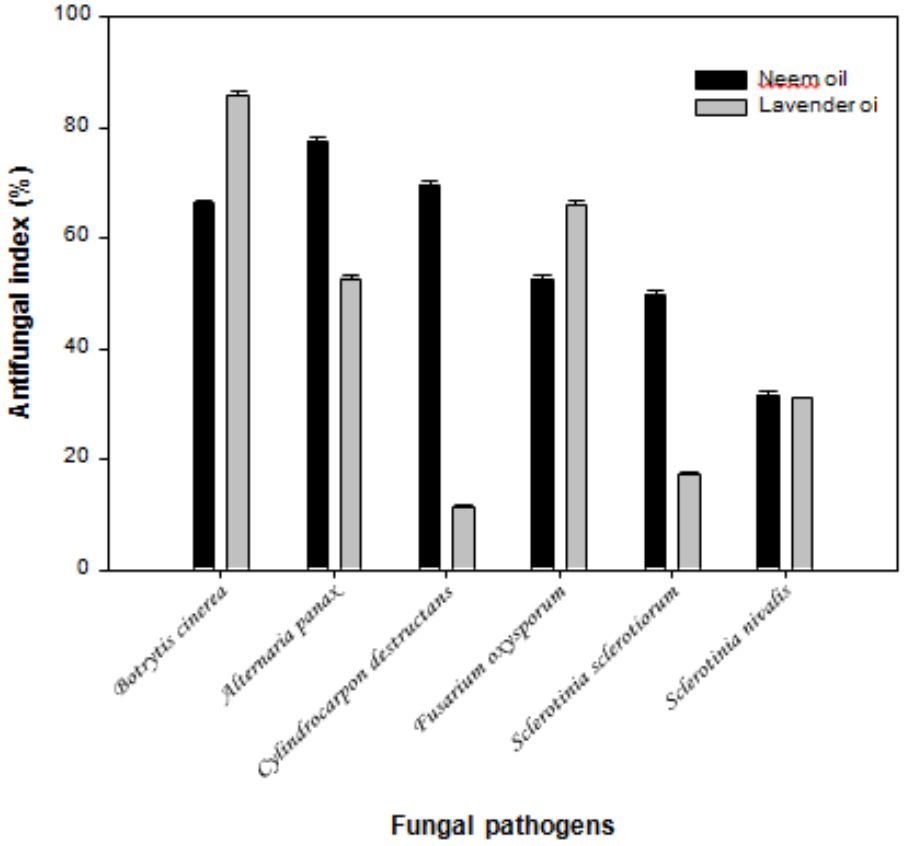

Figure 1. Antifungal activities of the essential oils neem (black bar) and lavender (grey bar) against ginseng root rot fungi at 10% v/v. The phytopathogenic fungi (agar dilution tests) were examined after 10 days and the inhibition percentages were calculated.

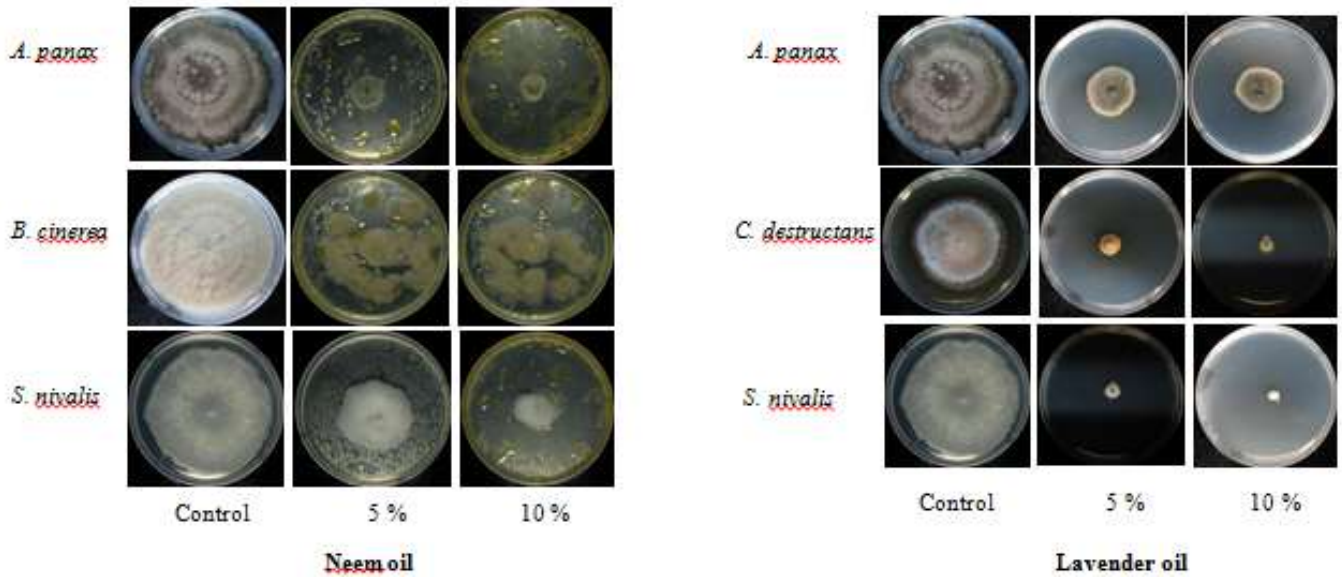

Figure 2. Antifungal activities of neem and lavender essential oils against the growth of fungal pathogens causing ginseng root rot, on agar dilution test, phytopathogenic fungi were examined after 10 days and the inhibition percentage was calculated according to the formula of Messgo-Moumene et al. (2015).

Table 2. Chemical compositions of neem essential oil.

Peak No.	Compound	Molecular formula	RT (min)	Concentration (%)
1	Silane, triethylfluoro-	$C_6H_{15}FSi$	8:44	3.96
2	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	33:23	3.5
3	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	33:37	0.87
4	Oleic Acid	$C_{18}H_{34}O_2$	33:58	3.64
5	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	34:14	1.39
6	Tetradecanoic acid	$C_{14}H_{28}O_2$	37:18	7.24
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	40:27	78.25
8	2-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]	$C_{22}H_{46}O_{12}$	40:40	1.15

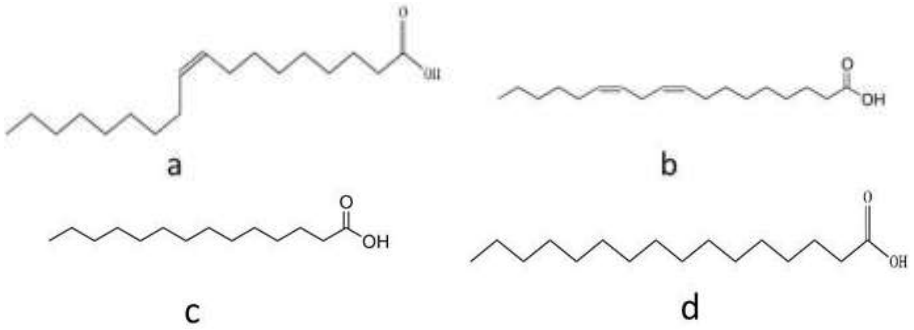

Figure 3. Main fatty acids skeletons of essential oil from neem (a) emery type, (b) linoleic type (c) myristic type (coconut oil) (d) palmitic type.

Table 3. Chemical compositions of lavender essential oil.

Peak No.	Compound	Molecular formula	RT (min)	Concentration (%)
1	Methanesulfonyl chloride	CH_3ClO_2S	6:57	5.67
2	Eucalyptol	$C_{10}H_{18}O$	7:03	5.99
3	Silane, triethylfluoro-	$C_6H_{15}FSi$	8:49	36.71
4	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (+)-	$C_{10}H_{16}O$	14:39	6.91
5	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	15:22	41.74
6	3-Cyclohexene-1-methanol, a,a4-trimethyl-	$C_{10}H_{18}O$	18:59	2.99

the main compound of essential oil (43.73%), followed by the octatriene dimethyl (25.10%), eucalyptol (7.32%) and camphor (3.79%). Some of the results in this study are in conformity with previous studies. The differences in chemical composition of plant essential oil may to some extent, due to the difference in maturity stages of growth, geographical sources and extraction techniques. Nevertheless, both investigated essential oils exhibited growth inhibition effect on the ginseng root rot fungal species tested in the present study. The results may assert the importance of essential oils of common plants in the preservation and protection of ginseng crop from fungal pathogens.

Conclusions

Searching for new antifungal agent from plant and detection of its ability to control plant diseases caused by phytopathogenic fungi are needed. *A. indica* (neem) and *L. angustifolia* (lavender) are among the most common plants used traditionally all over the world. The results on these plants showed their ability to be new sources of natural products used as antimicrobial agents. The results showed that their oils are active against the phytopathogenic fungal strains and opens the possibility for discovery of alternative clean method to control ginseng root rot.

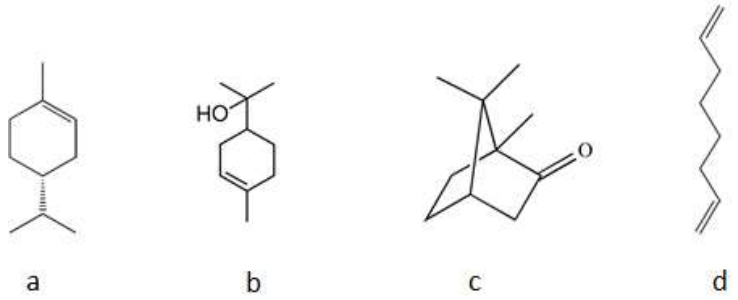

Figure 4. Main sesquiterpenes skeletons of essential oil from lavender (a) terpene type, (b) terpineol type, (c) camphore type, (d) octadien type.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project no. PJ010848)" Rural Development Administration, Republic of Korea.

REFERENCES

Adams RP (1995). Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured Publishing Corporation 469p.

Atabani AE, Silitonga AS,Ong HC,Mahlia TMI, Masjuki HH, Irfan AB, Fayaz H (2013). Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. Renew. Sustain. Energy Rev. 18:211-245.

Baeg IH, So SH (2013). The world ginseng market and the ginseng (Korea). J. Ginseng Res. 37:1-7.

Dandlen SA, Lima A, Sofia M, Marta D, Miguel M, Graça FM, Sousa L,

Pedro MJ, Barroso LG, Figueiredo JG, Cristina A (2011). Antimicrobial activity, cytotoxicity and intracellular growth inhibition of Portuguese Thymus essential oils. Revista Brasileira de Farmacognosia, 21(6):1012-1024.

Davis J, Persons W S (2014). Growing and Marketing Ginseng, Goldenseal and other Woodland Medicinals. Printed in Canada. First printing June

Djenontin TS, Wotto VD, Avlessi F, Lozano P, Sohounhloue DKC, Pioch D (2012). Composition of *Azadirachta indica* and *Cara paprocera* (Meliaceae) seed oils and cakes obtained after oil extraction. Ind. Crops Prod. 38:39-45.

Domsch KH, Gams W, and Anderson T (1980). Compendium of soil fungi. Academic Press. London 1-2:405-859.

Hui L, He L, Huan L, Xiao L, Aiguo Z (2010). Chemical composition of *lavender* essential oil and its antioxidant activity and inhibition against rhinitisrelated bacteria. African J. Microbiol. Res. 4:309-313.

Hyldgaard M, Mygind T, Meyer RL (2012). Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. Front Microbiol. 3:1-24.

Kamal J, Devi N, Thakur N, Tomar S, Shalit L, Thakur R (2012). *Trachyspermum ammi* (Ajwain): a comprehensive review. Int. Res. J. Pharm. 3:133-138.

Kocić-Tanackov SD, Dimić GR, Tanackov IJ, Pejin DJ, Mojović LjV, Pejin JD (2012). Antifungal activity of Oregano (*Origanum vulgare* L.) extract on the growth of *Fusarium* and *Penicillium* species isolated from food. Hemijska Industrija 66:33-41.

Lang G, Buchbauer G (2012). A review on recent research results on essential oils as antimicrobials and antifungals. A review. Flavour. Frag. J. 27:13-39.

Messgo-Moumene S, Li Y, Bachir K, Houmani Z, Bouznad Z, Chemat F (2015). Antifungal power of Citrus essential oils against potato late blight causative agent. J. Essen. Oil Res. 27:169-176.

Mondall NK, Mojumdar A, Chatterje SK, Banerjee A, Datta JK, Gupta S (2009). Antifungal activities and chemic al characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. J. Appl. Sci. Environ. Manage. 13(1):49-53.

Moubasher AH (1993). Soil fungi in Qatar and other Arab countries. The Centre of Scientific and Applied Research, University of Qater, Doha, Qater.

Oussalah MS, Caillet L, Saucier M L (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control 18:414-420.

Piyo A, Udomsilp J, Khang-Khun P, Thobunluepop P (2009). Antifungal activity of essential oils from basil (*Ocimum basilicum* Linn.) and sweet fennel (*Ocimum gratissimum* Linn.): Alternative strategies to control pathogenic fungi in organic rice. Asian J. Food Ag-Ind. (special issue), S2-S9.

Sandanasamy J, Nour AH, Bin Tajuddin SN, Nour AH (2013). Fatty acid composition and antibacterial activity of neem (*Azadirachta indica*) seed oil. The Open Conference Proceedings Journal. 4:43-48.

SAS Institute (2011). The SAS 10.2 software. Statistical Analysis System for Windows [Softaware]. Cary, N.C. SAS.

Schnitzler PA, Reichling J (2011). Screening for antiviral activities of isolated compounds from essential oils. 253643 J. Evid. Based. Complementary. Altern. Med. 25:36-43.

Seifert KA, McMullen CR, Yee D, Reeleder RD, Dobinson KF (2013). Molecular Differentiation and Detection of Ginseng-Adapted Isolates of the Root Rot Fungus *Cylindrocarpon destructans* Population Biology 93:1533-1542.

Shishtar E, Sievenpiper JL, Djedovic V, Cozma AI, Ha V, Jayalath VH (2014). The Effect of Ginseng (The Genus *Panax*) on Glycemic Control: A systematic review and meta-analysis of randomized controlled clinical trials. PLoS ONE 9(9): e107391.

Silva F S, Duarte A, Mendonça DI, Domingues FC (2011). Antifungal activity of *Coriandrum sativum* essential oil, its mode of action against candida species and potential synergism with amphotericin B. Phytomedicine. 19:42-47.

Tabassum N, Vidyasagar GM (2013). Antifungal investigations on plant essential oils: A review. Int. J. Pharm. Pharm. Sci. 5:19-28.

Wang SY, Wu CL, Chu FH, Chien SC, Kuo YH, Shyur LF, Chang ST (2005). Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. Wood. Holzforschung. 59:295-299.