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In vitro Inhibitory Activities of Essential Oils from *Oenanthe javanica* DC against *Candida* and *Streptococcus* species

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Abstract – The composition of essential oil from *O. javanica* was analyzed by gas chromatography-mass spectrometry. Using the broth dilution method and disk diffusion test, anti-microbial activities of the oil fraction and its main components were evaluated against various antibiotic-susceptible and resistant strains of pathogenic microorganisms. As a result of GC-MS analysis, 57 compounds, including α-terpinolene (28.1%), dl-limonene (16.0%), γ-terpinene (10.3%), β-pinene (9.7%) and α-pinine (6.0%) were identified in the essential oil fraction. The essential oil fraction of *O. javanica* and its main components exhibited significant inhibitory activities, particularly against *Candida albicans* (antibiotic-susceptible strains) and *Streptococcus pneumoniae* (antibiotic-susceptible and resistant strains). The main components of the *O. javanica* oil fraction displayed different patterns of activity against the three tested *Candida* species as exemplified by the differential minimum inhibiting concentration (MIC) values. The disk diffusion test showed that the activities were dose dependent.
Keywords – *Oenanthe javanica*, essential oil, antifungal, *Candida albicans*, *Streptococcus pneumoniae*

Introduction

Oenanthe javanica (wild-type), a favorite wild vegetable among Koreans, has a unique fragrance and flavor due to its characteristic essential oil content (Rhee *et al.*, 1995). The vegetable, which is collected in the spring in Korea, has additionally hemostatic, hematinic and diuretic activities, and has sometimes been employed as a treatment for pneumonia in folk medicine.

Essential oils from plants are still a promising source of novel natural anti-microbial agents, although their activities are generally milder than those of synthetic antifungal drugs (Hammer *et al.*, 2000; Giordani *et al.*, 2001; Shin and Kang, 2003). In particular, they show promise as an effective solution against rapidly emerging drug-resistant pathogens, which are raising the spectra of untreatable diseases (Girmenia *et al.*, 2003; Shin, 2003; Benincasa *et al.*, 2004; Shahidi-Bonjar, 2004). These oils may additionally act as substitutes for commercially available antibiotics, which are now mostly impotent against certain microbacterial infections in immunosuppressed diseases, including AIDS (Slevin *et al.*, 1981; May *et al*, 1995).

In this study, we analyzed essential oils from *O. javanica* and screened them for anti-microbial activity against selected pathogenic gram-positive and gram-negative bacteria, and fungi. In particular, growth-inhibition of *Streptococcus pneumoniae* and three common pathogenic *Candida* species was evaluated using the broth dilution method and disk diffusion test.

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Materials and Methods

Analysis of essential oils from *O. javanica* – Essential oils were obtained from the aerial parts of *O. javanica* cultivated in the province of Naju by steam distillation for five hours in a simultaneous steam distillation-extraction (SDE) apparatus. Essential oil fractions were analyzed by GC-FID and GC-MSD (Agilent 5973 network mass selective detector, 280°C) and the data obtained was compared with standards. Also, a Wiley 275 database search was performed on the data obtained from the analysis of the essential oils on a Hewlett-Packard 6890 GC system and a 5973 MSD apparatus equipped with a fused silica capillary column (HP-5 MS 5% phenyl methyl siloxane, 30 m×250 μm×0.25 μm), respectively. The injector was adjusted to 250°C and the oven temperature was set as follows: initial temperature: 50°C(isothermal, 5 min), increased to 2°C/min to 180°C, 5 min at 180°C, and 180-220°C (20°C/min). Oil standards and ketoconazole were purchased from Sigma Korea Ltd.

Strains – The tested strains tested were obtained from

the Culture Collection of Antibiotic Resistant Microbes (CCARM) and the Korean Culture Center of Micro-oganisms (KCCM).

The turbidity of the cell suspension was measured at 600 nm and adjusted with medium to match the 0.5 McFarland standard (10^5-10^6 colony forming units (CFU) ml^{-1}).

Screening of antimicrobial activities – Pathogenic organisms were screened for susceptibility to *O. javanica* essential oil by performing the disk diffusion test on cation-adjusted Mueller Hinton agar plates and trypticase agar plates with 5% sheep blood, according to the method of Performance Standards for Antimicrobial Susceptibility Testing (M100-S14) of NCCLS (National Committee of Clinical laboratory Standard).

MIC (minimum inhibiting concentration) test – MIC values of the oils were determined by the broth microdilution method. A range of two-fold dilutions (160-0.125 mg/ml) of essential oils in medium containing 2% of Tween-80 was prepared. The suspensions (100 μl) were then added to the wells of 96-well plates. Ketoconazole was similarly diluted in DMSO to generate a series of concentrations, ranging from 100 to 0.78 μg/ml per well. Next, 100 μl of the prepared broth culture of *Candida albicans*, *C. tropicalis* or *C. utilis*, was inoculated into each well and the plates were incubated at 25°C for 72 hours. MIC values were determined in duplicate and were re-examined when the values differed. Each organism was also cultured with a blank solution containing Tween 80 at concentrations equivalent to those in the test solutions to certify that these vehicles did not affect their growth.

Disk diffusion test – Fungal broth cultures of three *Candida* species obtained from KCCM were added to Sabouraud dextrose agar medium and distributed uniformly. Sterile paper discs (8mm) were wetted with 50 μl of each essential oil fraction of *O. javanica* (α-terpinolene, γ-terpinene, limonene, β-pinene, and α-pinene), placed on the culture plates, and cultivated at 25°C for 2-3 days. The diameters of the inhibited zones (mm) around the disks were measured.

Results and Discussion

Following steam distillation with an SDE apparatus and extraction with ether, an average of 0.34% (w/w) of essential oils was obtained from the aerial parts of *O. javanicum*.

As listed in Table 1, 57 compounds were identified in the essential oil fraction of this plant by GC-MS analysis. The most prominent compound was α-terpinolene, which

Table 1. Identified compounds in the essential oil fraction of *O. javanica*

Compounds	*Rt*	area (%)
heptanal	7.76	0.01
α-thujene	8.89	0.08
α-pinene	9.27	6.05
camphene	9.93	0.11
β-pinene	11.64	9.76
myrcene	12.64	2.86
α-phellandrene	13.25	0.88
γ-3-carene	13.54	0.02
α-terpinene	14.01	0.19
di-limonene	5.02	16.01
cis-ocimene	15.69	3.80
benzene acetaldehyde	16.00	0.09
γ-terpinene	17.00	10.34
α-terpinolene	19.14	28.10
l-linalool	19.94	0.16
1,3,8-para-menthatriene	20.33	0.05
2-cyclohexene-1-ol	21.16	0.03
terpinene-4-ol	24.88	0.34
cryptone	25.41	0.03
(−)-α-terpineol	25.97	0.19
carvacrol methyl ether	28.53	0.11
thymyl methyl ether	28.83	0.09
geraniol	30.52	0.01
(−)-bornyl acetate	32.02	0.17
trans-ocimene	32.70	0.01
α-ylangene	36.03	0.01
α-copaene	37.66	0.39
d-germacrene	37.90	0.07
γ-cadinene	38.44	0.05
β-cubebene	38.62	0.03
isocaryophyllene	39.58	0.02
β-caryophyllene	40.41	3.46
trans-α-bergamotene	41.52	0.07
α-humulene	42.43	1.57
trans-β-farnesene	43.06	0.40
α-selinene	43.81	0.03
α-amorphene	43.97	0.04
d-germacrene	44.15	1.49
β-selinene	44.43	0.07
bicyclogermacrene	45.10	1.32
(z)-cis-α-bergamotene	45.47	2.46
β-elemene	45.61	1.08
e, e-α-farnesene	46.27	1.90
δ-cadinene	46.91	3.68
α-calacorene	47.93	0.04
germacrene B	48.55	0.03
elemicin	49.27	0.04
nerolidol	49.52	0.41
(−)-caryophyllene oxide	50.10	0.22
ledene	50.28	0.01
viridiflorol	50.76	0.03
valerenol	50.94	0.01
γ-gurjunene	51.34	0.02
eremophilene	52.06	0.03
isospathylenol	53.49	0.01
α-cadinol	53.72	0.02
δ-elemene	53.74	0.02
In Total		98.52

comprised more than 28% of the oil. Other predominant compounds included dl-limonene (16.0%), γ-terpinene (10.3%), β-pinene (9.7%) and α-pinene (6.0%). Generally, non-oxygenated hydrocarbons comprised the majority of the oil fraction.

Among the tested pathogenic organisms, *Streptococcus pyrogens*, *S. pneumoniae* and *Candida albicans* exhibited the highest sensitivity to the essential oil fraction of *O. javanica*, resulting in more than a 30 mm growth inhibitory zone on agar culture plates in the disk diffusion test (Table 2). On the basis of these results, experiments were conducted using a ten-fold serially diluted oil suspension with culture medium containing Tween-80, with a view to determining the activity of this oil against various strains of *C. albicans* and *S. pneumoniae*. *S. pneumoniae* is the major cause of community-acquired bacterial pneumonia, and is commonly treated with penicillin (Koneman *et al.*, 1992; Baron *et al.*, 1994). For penicillin-allergic patients, erythromycin or other antibiotics, such as norfloxacin, are generally used. Approximately 5% of all *S. pneumoniae* strains are relatively resistant to penicillin (Teele, 2002). Moreover, *C. albicans* infection is often life-threatening due to the emergence of resistant strains during long-term or prophylactic treatment (Perieiro, 1997; Goldman et al., 2004).

As shown in Table 3, *O. javanica* oil displayed inhibitory activity against all the tested *C. albicans* and *S. pneumoniae* strains at concentrations ranging from 1:10 to 1: 1000000, revealing a significant deviation in sensitivity between strains. The most interesting finding from this experiment was that this oil additionally showed strong activity against most antibiotic resistant stains of *S. pneumoniae* tested.

Table 2. Screening of the volatile oil fraction of *O. javanica* for antimicrobial activity

	Strains	Inhibition zone* (mm)
Gram positive	*Streptoccus pyogens* 77A	>30
	Streptoccus pyogens ATCC8668	>30
	Streptoccus pneumoniae ATCC49619	>30
	Staphyloccus aureus SG511	11
	Staphyloccus aureus 285	20
	Staphyloccus aureus 503	11
	Staphyloccus aureus ATCC 29213	11
	Enterococcus faecalis ATCC29212	9
Gram negative	*Escherichia coli* 078	8
	Escherichia coli DC0	-**
	Escherichia coli 1507E	-
	Pseudomonas aeruginosa 9027	-
	Pseudomonas aeruginosa 1592E	11
	Pseudomonas aeruginosa 1771	-
	Pseudomonas aeruginosa 1771M	14
	Salmonella typhimurium	3
	Klebsiella oxytoca 1082E	8
	Klebsiella oxytoca 1552E	3
	Enterobacter cloacae P99	-
Fungi	*C. albicans* CCARM*14001*	>30

*The diameters of the inhibited zones (mm) around the disks were measured.
**No inhibition.

Table 3. Minimal dilution (MD) of the essential oil fraction of *O. javanica* required to inhibit various antibiotic-susceptible and resistant strains

Strains	M D	Susceptibility
C. albicans CCARM14001	1:10	ketoconazole susceptible
C. albicans CCARM14002	1:50	ketoconazole susceptible
C. albicans CCARM14003	1:10	ketoconazole susceptible
C. albicans CCARM14004	1:10	ketoconazole susceptible
C. albcians CCARM14020	1:10	ketoconazole susceptible
S. pneumoniae ATCC49619	1:100000	Penicillin, norfloxacin, erythromycin susceptible
S. pneumoniae CCARM4009	1:10000	Penicillin, norfloxacin, erythromycin resistant Norfloxacin resistant Erythromycin resistant
S. pneumoniae CCARM40101	1:1000000	Penicillin, norfloxacin, erythromycin resistant
S. pneumoniae CCARM40591	1:1000000	Penicillin, norfloxacin, resistant Erythromycin intermediately resistant
S. pneumoniae CCARM4070	1:10	Penicillin, norfloxacin, erythromycin resistant

Table 4. MIC* (mg/ml) of the volatile oil fraction (VOF) from *Oenanthum javanica* and its main components against *Candida* species

Fungi	α-terpinolene	γ-terpinene	limonene	β-pinene	α-pinene	VOF
C. albicans	64	64	16	64	16	32
C. tropicalis	32	64	8	16	4	16
C. utilis	8	16	8	16	8	8

* MIC values were determined in duplicate and were re-examined when the values differed.

Table 5. Growth inhibition*(mm) against *Candida* species on Sabouraud agar plates

Samples/disk		*C. albicans*	*C. tropicalis*	*C. utilis*
α-terpinolene	25mg	2.7±0.58	15.0±1.53	18.8±0.28
	50mg	4.5±0.50	19.0±2.08	2.3±1.53
γ-terpinene	25mg	1.8±0.29	2.2±0.29	2.5±0.87
	50mg	2.7±0.58	3.3±0.58	5.8±0.29
d, l-limonene	25mg	5.0±0.20	16.0±0.50	11.3±0.64
	50mg	5.8±0.76	19.0±2.00	17.0±0.00
β-pinene	25mg	1.2±0.76	4.7±0.58	5.7±1.53
	50mg	2.7±0.29	11.0±0.29	8.7±0.58
α-pinene	25mg	7.3±0.76	21.0±1.53	16.0±0.50
	50mg	10.5±1.32	31.0±0.58	29.0±1.53
VOF	25mg	2.0±0.00	2.7±1.53	3.3±0.29
	50mg	3.8±0.29	5.3±0.29	6.3±0.58
ketoconazole	50μg	0.5±0.00	15.0±2.00	1.8±0.29
	100μg	1.5±0.87	26.5±0.32	4.7±1.53

*Values (mm) are presented as means±SD of experiments performed in triplicate.
**VOF: volatile oil fraction of *O. javanica*.

Disk diffusion tests were performed to compare the activity of this oil on different *Candida* species. As shown in Table 4, the *O. javanica* oil fraction and its main components, β-pinene, α-pinene, α-terpinolene, γ-terpinene, and limonene, displayed distinct patterns of activity against the three tested *Candida* species as shown by their differential MIC values (ranging from 4 mg/ml to 64 mg/ml). Among the samples tested, limonene and a-pinene showed relatively stronger activity, with MIC values from 4 mg/ml to 16 mg/ml, compared to the other compounds tested. These results were confirmed by employing the disk diffusion test on Sabouraud agar plates using ketoconazole as control. The width of the inhibitory zone of fungal growth by the samples are listed in Table 5. The disk diffusion test revealed that the activities were dose-dependent.

In conclusion, the essential oil of *O. javanica* may be used as an effective agent against *Candida* and *S. pneumoniae* infections, and particularly against antibiotic-resistant strains of these organisms. Although its activity is much weaker than those of synthetic antibiotics, it is an attractive and effective agent against these pathogenic microorganisms, particularly in clinical situations where the unpleasant side-effects and toxicity of synthetic antibiotics limit their therapeutic use, such as in immunosuppressed patients (Eggimann *et al*, 2003; Shin, 2004). Further studies will be required to analyze the effects of the essential oil of *O. javanica* on drug-resistant *Candida* species.

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References

Baron, E.J., Peterson, L.R., and Finegold, S.M., Diagnostic Microbiology, Mosby, St Louis, P. 350 (1994).

Benincasa, M., Scocchia, M., Poddaa, E., Skerlavajb, B., Dolzanic, L., and Gennaro, R., Antimicrobial activity of Bac7 fragments against drug-resistant clinical isolates. *Peptides*, article in press (2004).

Eggimann, P; Garbino, J; Pittet, D., Epidemiology of Candida species infections in critically ill non- immunosuppressed patients. *Lancet Infectious Diseases* **3**, 685-702 (2003).

Girmenia, C., Venditti, M., and Martino, P., Fluconazole in combination with flucytosine in the treatment of fluconazole-resistant *Candida* infections. *Diagn. Micr. Infec. Dis.* **46**(3), 227-231 (2003).

Giordani, R., Trebaux, J., Masi, M., and Regli, P., Enhanced antifungal activity of ketoconazole by *Euphorbia characias* latex against *Candida albicans*. *J. Ethnopharmacol.* **78**, 1-5 (2001).

Goldman, G.H., Silva-Ferreira, M.E., Reis-Marques, E., Savoldi, M., Perlin, D., Park, S., Martinez, C.G., Goldman, M.H., and Colombo, A.L., Evaluation of fluconazole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in Brazil. *Diagn. Micr. Infec. Dis.* **50**, 25-32 (2004).

Hammer, K., Carson, C.F., and Riley, T.V., In vitro activities of ketoconazole, econazole, miconazole, and *Melaleuca alternifolia* (tea tree) oil against *Malassezia* species. *Antimicrob. Agents Ch.* **44**, 467-469 (2000).

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., and Winn, W.C., Diagnostic Micrbiology, J.B., Lippincott Company, p. 437 (1992).

May, T., Dailloux, M., Amiel, C., Laurain C., Canton, P., and Faou, A.L., Mycobacterial infections in AIDS patients; Lorraine, France. *J. Eur. Acad. Dermatol.* **4**, 100-103 (1995).

Perieiro, M., Losada, A., and Torbio, J., Adherence of *Candida albicans* strains isolated from AIDS patients. Comparison with pathogenic yeasts isolated from patients without HIV infection. *Brit. J. Dermatol.* **137**(1) 76-80 (1997).

Rhee, H.J., Koh, M.S., and Choi, O.J., A Study on the Volatile Constituents of the Water Dropwort (*Oenanthe javanica* DC) - according to extraction methods, parts and heating methods. *Korean J. Soc. Food Sci.* **11**, 386-395 (1995).

Shahidi-Bonjar, G.H., Inhibition of Clotrimazole-resistant *Candida albicans* by plants used in Iranian folkloric medicine. *Fitoterapia* **75**(1), 74-76 (2004).

Shin, S., and Kang, C.A., Antifungal activity of the essential oil of Agastache rugosa Kuntze and its synergism with ketoconazole. *Lett. Appl. Microbiol.* **36**, 111-115 (2003).

Shin S., Anti-*Aspergillus* activities of plant essential oils and their combination effects with ketoconazole or amphotericin B. *Arch. Pharm. Res.* **26**, 389-393 (2003).

Shin S., Essential oil compounds from Agastache rugosa as antifungal agents against *Trichophyton* species. *Arch. Pharm. Res.* **27**, 295-299 (2004).

Slevin, M.L., Lowes J.A., Bell, R., Catto-Smith, A.G., Ford, J.M., Malpas, J.S., and Lister; T.A., The role of transtracheal aspiration in the diagnosis of respiratory infection in neutropenic patients with acute Leukaemia. *Leukemia Res.* **5**, 165-168 (1981).

Teale, C.J., Antimicrobial resistance and the food chain. *J. of Appl. Microbiol.* **92**(1), 85-89 (2002).

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