Inhibitory effect of Plant Essential Oils on *Malassezia pachydermatis*

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Effect of the plant essential oils on the growth of *Malassezia pachydermatis* was evaluated and the essential oils of *Ocimum basilicum* L., *Melaleuca alternifolia* (Maid. & Bet.) Cheel, and *Rosa damascene* Mill. were the most active against *M. pachydermatis* and their activity were high than that of itraconazole at 2 mg/mL. The major constituents of the three oils by GC–MS analysis were linalool (21.83%) and estragole (74.29%) for *O. basilicum*, ã-terpinolene (17.96%) and terpinen-4-ol (45.54%) for *M. alternifolia*, and â-citronellol (59.98%) and geraniol (27.58%) for *R. damascene*. Results showed that these selected three oils could be effective toward controlling *M. pachydermatis* opportunistic infections.

Keywords: anti-*Malassezial* activity, essential oil, GC-MS analysis, *Malassezia pachydermatis*

The yeasts of the *Malassezia* genus are common commensal organisms of the skin of humans, birds and many domestic and wild animal species [Crespo *et al.*, 2002; Coutinho *et al*, 2006]. Today 10 lipid-dependent species are known (i.e. *M. dermatis, M. equi, M. furfur, M. globosa, M. japonica, M. nana, M. obtusa, M. restricta, M. slooffiae and M. sympodialis*), together with *M. pachydermatis* which is not dependent on lipid supplementation for *in vitro* growth [Hirai *et al.*, 2004]. In humans, *M. pachydermatis* was the responsible agent of infection both in immunocompetent [Ming Fan *et al.*, 2006], and in immunocompromised subjects [Midgley, 2000].

Yeasts are considered to be normal constituents of the feline ear microflora [Claudia *et al.*, 2005]. However, the pathogenic role of these yeasts has been recognized in various animals, mainly otitis externa and dermatitis disorders [Guillot and Bond, 1999; Crespo *et al.*, 2002].

Azoles, such as ketoconazole and itraconazole, are antifungal agents available in clinical practice, but they are highly toxic and expensive to use in prolonged treatments [Martinez Fernandez *et al.*, 1998]. Therefore, the development of more economical antifungal agents is required.

In this communication, we have analysed the antifungal properties of plant essential oils on *Malassezia pachydermatis*. The most active *Chamomilla recutita* (L.) Rauschert and *Artemisia dracunculus* L. essential oils were phytochemically examined by GC-MS analysis, and its main constituents were identified.

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In our experiments, 87 plant essential oils were used for antifungal activity tests, and they were purchased from UNIQ F&F Co., Ltd. (Seoul, Korea). The detailed information of these essential oils is shown in Table 1. Itraconazole was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were a reagent grade.

Malassezia pachydermatis (KCCM 50374) was obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). This strain was grown on Sabouraud Dextrose Broth (SDB) or Sabouraud Dextrose Agar (SDA) (Difco, Sparks, MD, USA) supplemented with 1% (v/v) of pure olive oil (Yakuri Pure Chemicals, Kyoto, Japan), following incubation at 37°C during 2-7 days. *Malassezia* strains were maintained on the same medium described previously, at 4°C, with subcultures being carried out on a monthly basis. The same medium was used in all the experiments. Inoculum suspensions were prepared by the method as described previously [Rukayadi *et al.*, 2006]. One milliliter of 48 h culture was centrifuged (3000 g at 4°C for 1 min), followed by washing the pellets twice with 1 mL of phosphate buffered saline (PBS). Clusters of *Malassezia* cells were formed upon preparation of inoculum suspensions. The washing of these suspensions with PBS promotes single-cell status and more accurate turbidity measurements.

The antifungal activity of the plant essential oils was carried out by the disk diffusion method [Anesini and Perez, 1993] using 100 μL of suspension containing 5×10^6 CFU/mL of *M. pachydermatis*. The disks (Whatman, 6 mm in diameter) which impregnated with 2, 1.5, 0.5, and 0.1 mg/mL of essential oil were placed on the inoculated agar. Control disk containing only ethanol employed to dissolve the essential oil showed no inhibition. Itraconazole of four concentrations were used as positive reference standards. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

The analysis was performed by gas chromatography-mass spectrometry (6890, Aglient Co., Palo Alto, CA, USA). Helium as carrier gas for individual constituents was averaged. The amount of the samples injected was 0.2 µL in split mode (400:1). The injector temperature was set at 270°C. The GC column was DB- 5MS stationary phase (60 m×0.32 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). The GC oven temperature was initially maintained at 60°C for 2 min and then programmed to 5°C/min to 300 and maintained for 5 min. 0.2 µL of sample dissolved in CH_2Cl_2 (1:100 v/v) was injected. Essential oil samples were analyzed and the relative peak areas for individual constituents averaged. Quantification was computed as the percentage contribution of each compound to the total amount present. The percentage composition of the oils was computed by the normalization method from the GC peak areas. GC-MS analysis of the essential oil with the GC (6890 Plus, Agilent Co.) coupled with 5973 mass selective detector quadrupole mass spectrometer. The mass spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV. The mass spectrometer was operated in full scan mode between 35 and 700 amu. The components of essential oil were tentatively identified by comparison of mass spectra of each peak with those of authentic samples in the NIST MS library.

Antifungal activity of 86 plant essential oils was found when the essential oil was assayed at 2 mg/mL (Table 1). The results showed the inhibitory effects of 11 oils [*Ocimum basilicum* L.,

Table 1. Antifungal activities against *Malassezia pachydermatis* of 86 plant essential oils

Oil	Family	Species	Part	Antifungal activity (cm)
Cananga	Annonaceae	*Cananga odorata* Hook fil. et Thomp.	flower	-
Ylang ylang	Annonaceae	*Cananga odorata* Hook. f. et Thomson	flower	-
Anise	Apiaceae	*Pimpinella anisum* L.	fruit	-
Aniseed	Apiaceae	*Pimpinella anisum* L.	fruit	-
Carrot seed	Apiaceae	*Daucus carota* L.	seed	-
Celery seed	Apiaceae	*Apium graveolens* L.	seed	-
Coriander	Apiaceae	*Coriandrum sativum* L.	flower	-
Fennel	Apiaceae	*Foeniculum vulgare* Mill.	seed	-
Galbanum	Apiaceae	*Ferula galbaniflua* Boiss.et Buhse	root	-
Lovage root	Apiaceae	*Levisticum officinale* L. Koch	fruit	-
Parsley herb	Apiaceae	*Petroselinum crispum (*Mill.*)* Nyman	whole plant	-
Parsley seed	Apiaceae	*Petroselinum crispum (*Mill.*)* Nyman	seed	-
Star anise	Apiaceae	*Illicium verum* L.	fruit	-
Armoise	Asteraceae	*Artemesia vulgaris* L.	whole plant	-
Chamomile blue	Asteraceae	*Chamomilla recutita* (L.) Rauschert	flower	-
Chamomile roman	Asteraceae	*Chamaemelum nobil* (L.) All.	flower	-
Davana	Asteraceae	*Artemisia pallens* Wall. Ex DC	whole plant	-
Helichrysum	Asteraceae	*Helichrysum angustifolium* DC	flower	-
Estragon	Asteraceae	*Artemisia dracunculus* L.	leaf	-
Wormwood	Asteraceae	*Artemisia absinthium* L.	flower	-
Frankincense	Burseraceae	*Boswellia thurifera* Roxburgh	root	-
Myrrh	Burseraceae	*Commiphora myrrha* var. *molmol* Engl.	stem	-
Tarragon	Asteraceae	*Artemisia dracunculus* L.	stem	-
Yarrow	Asteraceae	*Achillea millefolium* L.	flower	-
Cade	Cupressaceae	*Juniperus oxycedrus* L.	wood	-
Cedarleaf	Cupressaceae	*Thuja occidentalis* L.	leaf	-
Cedarwood	Cupressaceae	*Juniperus irginiana* L.	bark	-
Cedarwood Chinese	Cupressaceae	*Juniperus funebris* Endl.	bark	-
Cedarwood Texas	Cupressaceae	*Juniperus mexicana* Spring.	bark	-
Cypress	Cupressaceae	*Cupressus sempervirens* L.	twig	-
Juniperberry	Cupressaceae	*Juniperus communis* L.	berry	-
Wintergreen	Ericaceae	*Gaultheria procumbens* L.	leaf	-
Cascarilla bark	Euphorbiaceae	*Croton eleuteria* Bennett	bark	-
Basil	Lamiaceae	*Ocimum basilicum* L.	flower	1.5
Basil sweet	Lamiaceae	*Ocimum basilicum* L.	whole plant	-
Clary sage	Lamiaceae	*Salvia sclarea* L.	flower	-
Hyssop	Lamiaceae	*Hyssopus officinalis* L.	leaf	-
Lavender	Lamiaceae	*Lavaendula officinalis* (Chaiz.)	flower	-
Lavender 10/42	Lamiaceae	*Lavandula angustifolia* Mill.	flower	-

Table 1. Continued.

Oil	Family	Species	Part	Antifungal activity (cm)
Marjoram	Lamiaceae	*Thymus mastichina* L.	leaf	-
Melissa	Lamiaceae	*Melissa officinalis* L.	leaf	-
Patchouly	Lamiaceae	*Pogostemon cablin* (Blanco) Benth.	leaf	1.2
Peppermint	Lamiaceae	*Mentha piperita* L.	flower	-
Rosemary	Lamiaceae	*Rosmarinus officinalis* L.	flower	-
Sage	Lamiaceae	*Salvia officinalis* L.	whole plant	-
Sage Dalmatian	Lamiaceae	*Salvia officinalis* L.	leaf	-
Sage Spanish	Lamiaceae	*Salvia lavandulaefolia* Vahl.	leaf	-
Spearmint	Lamiaceae	*Mentha spicata* L.	flower	-
Cassia especial	Lauraceae	*Cinnamomum cassia* Bl.	bark	-
Cinnamon bleached	Lauraceae	*Cinnamomum zeylanicum* Garc. Ex Blume Nees	bark	-
Cinnaom leaf oil terpenes	Lauraceae	*Cinnamomum zeylanicum* Garc. Ex Blume Nees	leaf	-
Rosewood	Lauraceae	*Aniba roseadora* var. amazonica Ducke	wood	-
Nutmeg	Myristicaceae	*Myristica fragrans* Houtt.	seed	-
Eucalyptus	Myrtaceae	*Eucalyptus globulus* Labill.	leaf	-
Eucalyptus 80/85	Myrtaceae	*Eucalyptus globulus* Labill.	leaf	-
Myrtle	Myrtaceae	*Myrtus communis* L.	leaf	-
Niaouli	Myrtaceae	*Melaleuca viridiflora* Sol. Ex Gaertn.	leaf	-
Tea tree	Myrtaceae	*Melaleuca alternifolia* (Maid. & Bet.) Cheel	leaf	1.5
Jasmin absolute	Oleaceae	*Jasminum grandiflorum* L.	flower	-
Pine	Pinaceae	*Pinus sylvestris* L.	needle	-
Pine needle	Pinaceae	*Pinus sylvestris* L.	needle	-
Black pepper	Piperaceae	*Piper nigrum* L.	fruit	-
Geranium	Poaceae	*Pelargonium graveolens* L.	flower	0.9
Lemongrass	Poaceae	*Cymbopogon citratus* (DC) Stapf.	whole plant	1.2
Vetiver Haiti	Poaceae	*Vetiveria zizanioides* L.	root	-
Bergamot	Rutaceae	*Citrus bergamia* Risso	peel	-
Buchu	Rutaceae	*Agathosma crenulata* (L.) Pillans	leaf	-
Buchu leaf	Rutaceae	*Agathosma betulina* (Berg.) Pillans	leaf	-
Grapefruit	Rutaceae	*Citrus paradisi* Macfadyen	fruit	0.9
Orange	Rutaceae	*Citrus sinensis* (L .) Osbeck	peel	-
Lemon	Rutaceae	*Citrus limonum* L.	peel	-
Lemon 10F	Rutaceae	*Citrus limonum* L.	peel	-
Lime dis 5F	Rutaceae	*Citrus aurantifolia* Swing.	peel	-
Mandarine	Rutaceae	*Citrus reticulata* Blanco	peel	-
Neroli	Rutaceae	*Citrus aurantium* L.	flower	-
Petitgrain	Rutaceae	*Citrus aurantium* L. subp. amara	leaf	-
Tangerine	Rutaceae	Citrus reticulata Blanco	peel	-
Sandalwood	Santalaceae	*Santalum album* L.	wood	0.9
Valerian	Valerianaceae	*Valeriana officinalis* L.	rhizome	-
Rose	Rosaceae	*Rosa damascene* Mill.	flower	1.8
Chamomile blue	Asteraceae	*Chamomilla recutita* (L.) Rauschert	flower	1.4
Estragon	Asteraceae	*Artemisia dracunculus* L.	leaf	1.2
Tamanu	*Clusiaceae*	*Calophyllum inophyllum* L.	fruit	-
Xanthoxylum	Rutaceae	Zanthoxylum armatum	seed	-
Eucalyptus	Myrtaceae	Eucalyptus citriodora	leaf	0.9
Ginger	Zingiberaceae	*Zingiber officinale* Roscoe	rhizome	-
Itraconazole				1.5

Samples treated with concentration of 2 mg. '-' expressed no activity.

Pogostemon cablin (Blanco) Benth., *Melaleuca alternifolia* (Maid. & Bet.) Cheel, *Pelargonium* graveolens L., *Cymbopogon citratus* (DC) Stapf., *Citrus paradisi* Macfadyen, *Santalum album* L., *Rosa damascene* Mill., *Chamomilla recutita* (L.) Rauschert, *Artemisia dracunculus* L., and *Eucalyptus citriodora*] on *M. pachydermatis* at 2 mg/mL. Among the 11 active oils, the oils of *Ocimum basilicum* L., *Melaleuca alternifolia* (Maid. & Bet.) Cheel, and *Rosa damascene* Mill. showed strong inhibitory activity at a dose dependant manner and the activity were higher than itraconazole at the same concentration (Fig. 1).

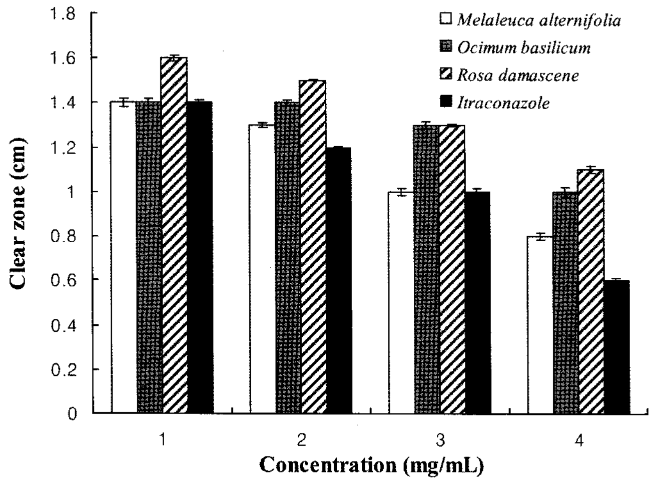

Fig. 1. Antifungal activity of essential oil of *Ocimum basilicum*, *Melaleuca alternifolia*, and *Rosa damascene* against *Malassezia pachydermatis*. Values represent the means of 3 independent experiments.

Table 2. Chemical composition of *Melaleuca alternifolia* essential oil

No.	Compound	RI [a]	Relative composition ratio, %
1	α-thujene	921	0.65
2	α-pinene	928	2.50
3	β-pinene	967	0.59
4	β-myrcene	981	6.44
5	α-terpinene	1,007	7.25
6	*p*-cymene	1,010	5.32
7	1,8-cineole	1,018	3.72
8	Limonene	1,019	0.99
9	γ-terpinene	1,049	17.96
10	Terpinolene	1,077	2.81
11	Terpinen-4-ol	1,159	45.54
12	α-terpineol	1,170	3.26
13	Armodendrene [b]	1,435	1.02
14	Cadinene [b]	1,514	0.88
Total			98.93

[a]Retention indices.
[b]Tentatively identified by mass library.

Table 3. Chemical composition of *Ocimum basilicum*

No.	Compound	RI [a]	Relative composition ratio, %
1	Linalool	1,084	21.83
2	Estragole	1,173	74.29
3	α-Humulene [b]	1,447	2.17
Total			98.29

[a]Retention indices.
[b]Tentatively identified by mass library.

The three oils were analysed by GC-MS. The chemical compositions of *O. basilicum* oil are shown in Table 2~4. Altogether 3 compounds were identified, representing 98.29% of the total oil constituents. The major constituents of the oil were linalool (21.83%) and estragole (74.29 %). *M. alternifolia* oil composed of major constituents of the γ-terpinolene (17.96%) and terpinen-4-ol (45.54%). The major constituents of *R. damascene* were β-citronellol (59.98%) and geraniol (27.58%).

Table 4. Chemical composition of *Rosa damascene*

No.	Compound	RI [a]	Relative composition ratio, %
1	Linalool	1,084	3.56
2	Roseoxide	1,096	1.72
3	β-Citronellol	1,211	59.98
4	Nerol [b]	1,214	1.75
5	Geraniol	1,236	27.58
6	2-Phenylethyl acetate [b]	1,242	2.04
7	Geranyl acetate	1,360	0.75
8	Methyl eugenol	1,370	1.24
	Nonadecane [b]	1,872	1.39
Total			100.0

[a]Retention indices.
[b]Tentatively identified by mass library.

Consequently, we demonstrated that the 11 oils among the 86 plant essential oils had inhibitory activity against *M. pachydermatis* at first screening. The highest antifungal activity was found in the essential oils of *O. basilicum*, *M. alternifolia*, and *R. damascene*. This activity was dose-dependant and higher than that of itraconazole. Although, further studies are needed, the use of essential oils of *O. basilicum*, *M. alternifolia*, and *R. damascene* against microbial growth seems a valuable alternative as antifungal compound, especially in the cases of anti-*Malassezia* resistance.

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