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Vapor activity of 72 essential oils against a *Trichophyton mentagrophytes*

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Abstract For practical application for vapor therapy and fumigation, the vapor activity of 72 essential oils was screened against a *Trichophyton mentagrophytes*, using a closed box. The fungicidal activity, expressed as the minimum fungicidal dose (MFD), was determined from the colony size, which was correlated with the inoculum size. Oils containing phenol as the major component showed the most potent vapor activity, with an MFD of 1.56 μg/ml air. This was followed by oils with aldehyde as the major component, then by those with alcohol as the major component. The vapor activities of oils containing ketone, ester, and ether/oxide components were decreased, in that order. The oils that were rich in hydrocarbon components had the weakest activity. The same tendency was observed with the components themselves. Phenols and aldehydes exhibited the highest vapor activity, followed by alcohols, ketone, ester, ether/oxide, and hydrocarbon. There was a rough correlation between the vapor activity determined by the box vapor assay and the contact activity determined by agar diffusion assay. But oils containing sesquiterpenes showed weaker vapor activity than that expected from the contact activity. Based on the activity-chemical structure relationship, the 72 oils were classified into seven functional groups.

Key words Essential oil · Anti-fungal activity · Chemical function · *Trichophyton mentagrophytes*

Different from many antimicrobial agents, essential oils show vapor activity against bacteria and fungi.[1] Recently, the vapor activity of these oils has attracted renewed attention as natural preservatives, owing to food-safety concerns and growing problems with microbial resistance in the food industry.[2–5] In the medical field, room disinfection,[6] the prevention of respiratory tract mycosis,[7] and the removal of methicillin-resistant *Staphylococcus aureus* (MRSA) from the human body and clothing[8] have been reported utilizing essential oils.

Our laboratory has been investigating the possible application of the vapor activity of these oils for the treatment of respiratory tract infections,[9] fungal infections,[10] and superficial fungal infections such as tinea pedis.[11] Treatment of such superficial infections with oil vapor has the advantage of being clean, because no carrier such as oil or gel is necessary, and uniform, because the vapor reaches a wide area of infection uniformly. In order to advance our project for practical application, we screened the vapor activity of 72 essential oils against a *T. mentagrophytes*, using the box vapor assay reported in a previous study.[12] The vapor activity was compared with the oils' contact activity, determined by an agar diffusion assay.

In regard to materials and methods, essential oils were obtained from Laboratoire Sanoflore, Gigots-et-Lozeron, France (via Hyper Plants, Tokyo, Japan), unless otherwise stated. Bergamot, German chamomile, cypress, ginger, hyssop, lemon eucalyptus, oregano, rosemary "vervenone," thyme geraniol, and valerian oils were obtained from Pranarom, International, Ghislenghien, Belgium (via Kenso Igakusha, Yamanashi, Japan). Cedarwood Himalaya, cinnamon bark, and sweet fennel oils were from La Florina, Lautertal, Germany (via Tennokaori, Tokyo, Japan). Perilla oil, a cold-pressed product, was from Kohken Koryo (Yokohama, Japan). Wild thyme was from Niels Yard Far East (Tokyo, Japan), and Japanese mint was from Nagaoka Jitsugyo (Nagaoka, Japan).

Santolina and tansy oils were prepared in this laboratory by steam distillation of the parent herbs; from the leaves of *Santolina chamaecyparissus* and the leaves and flowers of *Tanacetum vulgare*, respectively, cultivated at Chichibu, Japan. In the same way, lindera and eupatorium essential oils were prepared from the shrubs *Lindera umbellata* (which grows wild on Chichibu mountain), and *Eupatorium*

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laciniatum (which is cultivated at Hozenji temple, in Nagatoro), respectively. The herb, *Perilla frutescens* var. *japonica* (egoma) was cultivated at Chichibu farm of the Saitama Prefecture Agriculture and Forestry Research Center, Japan, and its essential oil was prepared from the leaves in this laboratory. The oil of *Laserpitium siler* was a gift from Laboratoire Sanoflore. Chemical compounds were obtained from Tokyo Kasei Kogyo (Tokyo, Japan), unless otherwise stated. Limonene, 3-carene, and β-caryophyllene were obtained from Nippon Terpene Chemical (Kobe, Japan). Perillaldehyde was obtained from Kohken Koryo (Yokohama, Japan), farnesol was from Wako Pure Chemical (Osaka, Japan), and cedrol was from Sigma (St. Louis, MO, USA).

A gas chromatography (GC) apparatus (model HP5890; Hewlett Packard, Palo Alto, CA, USA) coupled with a mass spectrometry (MS) apparatus (model HP5989; Hewlett Packard) was used. The temperature of the TC-5 column (0.25 mm × 30 m; GL Sciences) was raised from 60° to 200°C, at a rate of 5°C per min. GC peaks were identified by comparing the MS fragmentation pattern and relative retention time with those of the reference compounds available. The area of the GC peak was used for quantitative determination.

In regard to test organisms and culture media, *T. mentagrophytes* TIMM2789 was a stock culture of Teikyo University Institute of Medical Mycology. The small conidia of this strain were reproduced well. Sabouraud dextrose agar and 1.5% Bacto agar were used in the box vapor assay. Agar medium containing 1% peptone, 1% glucose, and 1% Agarose-1 was used in the agar diffusion assay.

A conidial suspension with a density of 1×10^8 conidia/ml was prepared according to the procedure reported previously.[12]

The box vapor assay against *T. mentagrophytes* TIMM2789 was carried out according to the procedure reported previously.[12] Three agar plugs implanted with a *T. mentagrophytes* at 10^7 cells/ml were placed, with the mycelial side on the top, on a petri dish, and placed in an airtight box (1.3-l air capacity). A twofold dilution series of an essential oil or pure compound dissolved in ethyl acetate was soaked uniformly on a filter paper (150 mm in diameter) and placed on the top of the box. An aluminum foil was inserted between the filter paper and the upper lid. The box was sealed and incubated for 24 h at 27°C.

The agar plugs treated were taken out, and placed, with the mycelial side on the top, on a square plate containing 1.5% Sabouraud dextrose agar. The plate was incubated at 27°C for 4 days. The average colony size of three plugs was obtained. The number of surviving mycelial cells was determined according to the standard curve, which was obtained from the correlation between the diameter of the colony and the number of conidia originally inoculated on the agar plugs at 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7/ml.[13] The vapor activity was expressed as the minimum fungicidal dose (MFD) per unit air space that killed more than 99.9% of the original inoculum.

Details of the procedures for the agar diffusion assay, using a paper disc, have been described previously.[12] That is, 30 µl of a 1%, 5%, or undiluted essential oil or pure compound dissolved in ethyl acetate was put on a disc, which was placed at the center of double-layered agar medium in a petri dish. The dish was sealed with vinyl tape and incubated at 27°C for 4 days. The inhibitory diameter was measured by means of a slide caliper. All the assays were performed in triplicate, and the mean values ± SD were recorded.

Table 1 shows the major components of the 72 essential oils (determined in this laboratory), and their antifungal activity, determined by the box vapor assay and the agar diffusion assay. Most of the compositions were close to those reported.[14,15] However, citronella oil contained geraniol as the major component, and the contents of citronellal and citronellol were very low, different from the literature. Sabinene and myrcene, reported in the literature, were not found in juniper berry oil. Tansy oil was found to belong to the t-chrysanthenyl acetate chemotype among various chemotypes reported. The essential oil of eupatorium was not listed in the literature, and was first prepared and analyzed in this laboratory. The oil contained methyl thymol, and dimethoxydurene and β-caryophyllene as the major components. The composition of laserpitium oil was first analyzed in this study, revealing perillaldehyde as the major component, similar to perilla (shiso) oil, and extra blue-colored azulene as a minor component.

The antimicrobial activity of eupatorium, laserpitium, lindera, tansy, and perilla (egoma) oils has not been evaluated before. Except for a few oils,[11] the anti-*Trichophyton* activity of essential oils, determined by vapor contact, was first done in this study.

The box vapor assay revealed that essential oils having phenol as the major component exhibited the most potent vapor activity. The MFD values of these 4 oils were between 1.56 and 3.13 µg/ml air, with an average of 1.56 µg/ml air. The most active oils were oregano and wild thyme . Six oils having aldehyde as the major component showed potent vapor activity, with an average MFD of 3.13 µg/ml air. The vapor activity was decreased in the order of oils containing alcohol, ketone and ester, and ether and oxide as the major components. Among the 21 oils with alcohol as the major component, citronella, coriander, geranium "Bourbon", lindera, palmarosa, peppermint, rosewood, thyme thujanol, and thyme geraniol were relatively potent, each showing an MFD of 6.25 µg/ml air. The average MFD of the alcohol oils was 12.5 µg/ml air. The vapor activity of the ketone oils was weaker than that of the alcohol oils. Among the 6 ketone oils, spearmint oil showed potent vapor activity (6.25 µg/ml air). The average MFD for the ketone oils was 25 µg/ml.

The oils containing ester as the major component showed much weaker vapor activity than the ketone oils. The average MFD of these 8 oils was 50 µg/ml air. Tansy oil was relatively active (12.5 µg/ml air), whereas black birch and wintergreen oils, consisting of methyl salicylate, were inactive. The vapor activity of the 12 oils containing ether/oxide as the major component was similar to that of the ester oils, showing an average MFD of 50 µg/ml air. Ravensara oil showed considerable activity (12.5 µg/ml air).

Table 1. Major components of 72 essential oils, and results of box vapor and agar diffusion assays of the oils against *T. mentagrophytes* TIMM2789

Essential oil (parent plant)	Major component[a]	Box vapor assay MFD (μg/ml air)[b]	Agar diffusion assay ID ± SD (mm)[c]
Phenol oils (4 oils)			
Clove (*Eugenia caryophyllata*)	Eugenol (87%)	3.13	45 ± 5
Oregano (*Oreganum vulgare*)	Carvacrol (26%), thymol (23%), γ-terpinene (22%)	1.56	>80
Thyme thymol (*Thymus vulgaris*)	Thymol (25%), limonene (26%)	3.13	55 ± 6
Wild thyme (*Thymus serpyllum*)	Carvacrol (80%)	1.56	>80
Aldehyde oils (6 oils)			
Cinnamon bark (*Cinnamonum zeylanicum*)	Cinnamaldehyde (54%)	3.13	>80
Laserpitium (*Laserpitium siler*)	Perillaldehyde (47%), limonene (39%), azulene (0.7%)[d]	6.25	53 ± 7
Lemon eucalyptus (*Eucalyptus citriodora*)	Citronellal (73%)	3.13	24 ± 4
Lemongrass (*Cymbopogon citrates*)	Citral (69%)	1.56	>80
Melissa (*Melissa officinalis*)	Citral (37%), β-caryophyllene (25%)	3.13	32 ± 6
Perilla (shiso) (*Perilla frutescens* var. *crispa*)	Perillaldehyde (66%)	6.25	75 ± 8
Alcohol oils (21 oils)			
Sweet basil (*Ocimum basilicum*)	Linalool (51%)	>50	10 ± 0
Cedarwood Virginia (*Juniperus virginiana*)	Cedrol (32%), α-cedrene (29%)	>100	13 ± 1
Citronella (*Cymbopogon nardus*)	Geraniol (20%)	6.25	25 ± 3
Coriander (*Coriandrum sativum*)	Linalool (71%)	6.25	14 ± 2
Geranium "Bourbon" (*Pelargonium roseum*)	Citronellol (26%), geraniol (13%)	6.25	55 ± 4
Japanese mint (*Mentha arvensis*)	Menthol (42%), menthone (23%)	25	13 ± 1
Spike lavender (*Lavandula latifolia*)	Linalool (46%), 1,8-cineole (23%)	25	0
True lavender (*Lavandula angustifolia*)	Linalool (30%), linalyl acetate (37%)	25	10 ± 0 (F)[e]
Lavandin (*Lavandula intermedia*)	Linalool (38%), linalyl acetate (27%)	12.5	10 ± 0
Lindera (*Lindera umbellata*)	Linalool (59%), geraniol (22%)	6.25	45 ± 8
Sweet marjoram (*Origanum marjorana*)	Terpinen-4-ol (25%)	12.5	28 ± 4
Neroli (*Citrus aurantium* var. *amara*)	Linalool (39%), limonene (16%)	25	18 ± 2
Palmarosa (*Cymbopogon maritinii* var. *martini*)	Geraniol (84%)	6.25	63 ± 3
Patchouli (*Pogostemon cablin*)	Patchoulol (42%)	>100	17 ± 2
Peppermint (*Mentha piperita*)	Menthol (45%)	6.25	9.5 ± 0 (F)
Rose otto (*Rosa damascena*)	Citronellol (28%), geraniol (17%)	12.5	35 ± 6
Rosewood (*Aniba rosaeodora*)	Linalool (83%)	6.25	45 ± 3
Sandalwood (*Santalum album*)	α-Santalol (50%), β-santalol (23%)	25	16 ± 3 (F)
Tea tree (*Melaleuca alternifolia*)	Terpinen-4-ol (43%)	25	11 ± 0
Thyme thujanol (*Thymus vulgaris*)	Thujanol (45%)	6.25	29 ± 5
Thyme geraniol (*Thymus vulgaris*)	Geraniol (30%), geranyl acetate (52%)	6.25	27 ± 2
Ketone oils (6 oils)			
Caraway (*Carum carvi*)	(+)-Carvone (57%)	12.5	25 ± 7
Hyssop (*Hyssopus officinalis*)	Isopinocamphone (30%), pinocamphone (20%)	25	12 ± 2
French lavender (*Lavandula stoechas*)	Fenchone (58%), camphor (20%)	50	0
Sage (*Salvia officinalis*)	α-Thujone (26%), 1,8-cineole (14%), camphor (13%)	50	16 ± 5
Santolina (*Santolina chamaecyparissus*)	Artemisiaketone (14%), vulgarone A (9%), B (8%)[d]	50	24 ± 3
Spearmint (*Mentha spicata*)	(−)-Carvone (55%)	6.25	11 ± 0 (F)
Ester oils (8 oils)			
Bergamot (*Citrus aurantium* subsp. *bergamia*)	Linalyl acetate (33%), limonene (35%)	100	0
Black birch (*Betula lenta*)	Methyl salicylate (100%)	>100	0
Roman chamomile (*Chamaemelum nobile*)	Isobutyl angelate (34%), isoamyl angelate (18%)	25	19 ± 4

Table 1. *Continued*

Essential oil (parent plant)	Major component[a]	Box vapor assay MFD (μg/ml air)[b]	Agar diffusion assay ID ± SD (mm)[c]
Clary sage (*Salvia sclarea*)	Linalyl acetate (69%)	100	10 ± 0
Helichrysum (*Helichrysum italicum*)	Neryl acetate (52%)	25	10 ± 0
Tansy (*Tanacetum vulgare*)	t-Chrysanthenyl acetate (69%)	12.5	14 ± 2
Valerian (*Valeriana officinalis*)	Bornyl acetate (38%), camphene (24%)	>50	11 ± 1
Wintergreen (*Gaultheria procumbens*)	Methyl salicylate (100%)	>100	0
Ether/oxide oils (12 oils)			
Tropical basil (*Ocimum basilicum*)	Methyl chavicol (92%)	100	11 ± 0
Cajeput (*Melaleuca cajeputii*)	1,8-Cineole (75%)	50	0
German chamomile (*Chamomilla recutita*)	Bisabolol oxide A (34%), β-farnesene (20%), α-bisabolol (12%)	>50	15 ± 6 (F)
Eucalyptus globulus (*Eucalyptus globulus*)	1,8-Cineole (68%)	25	0
Eucalyptus radiata (*Eucalyptus radiata*)	1,8-Cineole (65%)	25	0
Eupatorium (*Eupatorium laciniatum*)	Methyl thymol (23%), β-caryophyllene + dimethoxydurene (26%)	>50	26 ± 6
Sweet fennel (*Foeniculum vulgare*)	Anethole (78%)	100	9 ± 0
Myrtle (*Myrtus communis*)	1,8-Cineole (34%), myrtenyl acetate (23%)	50	9 ± 0
Niaouli (*Melaleuca viridiflora*)	1,8-Cineole (47%)	100	0
Perilla (egoma) *(Perilla frutescens* var. *japonica*)	Myristicin (59%)	>50	16 ± 5
Ravensara (*Ravensara aromatica*)	1,8-Cineole (50%)	12.5	9 ± 0 (F)
Rosemary "camphor" (*Rosmarinus officinalis*)	1,8-Cineole (32%), α-pinene (23%), camphor (9%)	25	19 ± 6
Hydrocarbon oils (15 oils)			
Black pepper (*Piper nigrum*)	β-Caryophyllene (29%), limonene (17%)	>100	0
Cedarwood Atlas (*Cedrus atlantica*)	β-Himachalene (36%), atlantone (21%)	100	11 ± 0
Cedarwood Himalaya (*Cedrus deodorata*)	β-Himachalene (36%), atlantone (10%)[d]	25	17 ± 5
Cypress (*Cupressus sempervirens*)	α-Pinene (60%)	>50	0
Frankincense (*Boswellia carteri*)	α-Pinene (31%), limonene (13%)	25	11 ± 0
Galbanum (*Ferula gummosa*)	3-Carene (68%), sesquiterpenols (12%)	>50	24 ± 1
Ginger (*Zingiber officinalis*)	Zingiberene (27%), β-bisabolene (16%)	>100	14 ± 2
Grapefruit (*Citrus paradisi*)	Limonene (93%)	200	11 ± 0
Juniper berry (*Juniperus communis*)	α-Pinene (35%), β-phellandrene (17%), β-pinene (12%)	>50	0
Lemon (*Citrus limonum*)	Limonene (66%)	>50	10 ± 0
Myrrh (*Commiphora myrrha*)	Lindestrene (38%), curzerene (27%), furanoeudesma-1,3-diene (11%)	25	20 ± 2
Nutmeg (*Myristica fragrans*)	α-Pinene (23%), myristicin (10%)	50	29 ± 1
Sweet orange (*Citrus sinensis*)	Limonene (95%)	>50	9.5 ± 0
Rosemary "vervenone" (*Rosmarinus officinalis*)	α-Pinene (31%), bornyl acetate (14%)	>50	0
Ylang ylang (*Cananga odorata*)	Germacrene D (20%), β-caryophyllene (17%)	>100	12 ± 2

[a] The major component is listed. When the content of the major component was <40%, the second and third major components, >10% content, were added
[b] MFD, minimum fungicidal dose
[c] ID ± SD, inhibitory diameter, including size of disc ± SD, when tested using 5% solution of essential oil
[d] A component of <10% content was listed exceptionally for reference in the text
[e] F means faint or unclear inhibition zone

Table 2. Box vapor assay and agar diffusion assay of essential oil components against *T. mentagrophytes* TIMM2789

Component	Functional chemical group	Box vapor assay MFD (μg/ml air)	Agar diffusion assay ID ± SD (mm)[b]
Carvacrol	Phenol	1.56	80 ± 0 (5%)
Eugenol	Phenol	1.56	23 ± 2 (1%)
Thymol	Phenol	3.13	41 ± 2 (1%)
Cinnamaldehyde	Aldehyde	1.56	56 ± 4 (1%)
Citral	Aldehyde	3.13	60 ± 5 (1%)
Perillaldehyde	Aldehyde	12.5	39 ± 5 (5%)
Linalool	Alcohol	12.5	11 ± 2 (5%)
Phenylethanol	Alcohol	12.5	12 ± 2 (5%)
Terpinen-4-ol	Alcohol	12.5	11 ± 1 (5%)
Farnesol	Sesquiterpene alcohol	>100	24 ± 9 (5%)
Cedrol	Sesquiterpene alcohol	>100	15 ± 4 (5%)
(−)-Carvone	Ketone	12.5	Spur (5%)
Camphor	Ketone	25	0 (5%)
Linalyl acetate	Ester	25	25 ± 3 (10%), 0 (5%)
Anethole	Ether	>100	Spur (5%)
1,8-Cineole	Oxide	50	13 ± 1 (10%), 0 (5%)
3-Carene	Hydrocarbon	>100	27 ± 3 (100%), 0 (10%)
Limonene	Hydrocarbon	100	53 ± 3 (100%), 0 (10%)
β-Caryophyllene	Sesquiterpene hydrocarbon	>100	0 (10%)

MFD, minimum fungicidal dose; ID ± SD, inhibitory diameter including size of disc ± SD; 0, no inhibition zone

The oils containing methylated phenol (methyl chavicol, methyl thymol, and anethole) as the major component were weak compared with the oils containing phenols. No vapor activity was observed in German chamomile, eupatorium, and perilla (egoma) oils. The oils in the hydrocarbon group showed the weakest activity. With the exception of cedarwood Himalaya, frankincense, and myrrh oils, many of these oils were very weak or inactive. The average MFD of these 15 oils was ⩾100 μg/ml air.

As judged from the mean MFD, the order of activity of the 72 oils was phenol oil > aldehyde oil > alcohol oil > ketone oil > ester oil = ether/oxide oil > hydrocarbon oil.

Table 2 shows the MFD values and inhibitory diameters of some of the oil components. The activity ranking was the same as that of the parent oils. Three phenol components and three aldehyde components exhibited the most potent activity by the box vapor assay. Three alcohols were ranked second, followed by two ketones, an ester, two ether/oxides, and three hydrocarbon components, in that order. Exceptionally, farnesol and cedrol showed no vapor activity.

Kalemba and Kunicka[16] suggested that the activity ranking of oil components by solution contact was phenol > aldehyde > ketone > alcohol > ether > hydrocarbon. The order of alcohol and ketone was reversed in our study. Our results suggested that the classification of essential oils into seven groups based on the functional groups of the major component may be justified for the assessment of their bioactivity. Furthermore, the classification seems to be reasonable, because the inhibitory activity of essential oils has been attributed to their most abundant components.[3,17–19] The synergistic interaction between individual components was reported to be too low to be of any practical importance.[20]

When the contact activity determined by the agar diffusion assay was compared with the vapor activity, there was a rough correlation between them, with some exceptions. Thus, phenol and aldehyde oils showed potent contact activity, in parallel to their potent vapor activity, except for lemon eucalyptus and melissa oils, which showed weaker contact activity. A close correlation of the two activities was observed for 13 of the 21 alcohol oils, 5 of the 6 ketone oils, 7 of the 8 ester oils, 5 of the 12 ether/oxide oils, and 13 of the 15 hydrocarbon oils.

Discrepancies between the vapor and contact activities were seen in two directions. One direction was that some oils showed weak contact activity by the agar diffusion assay but strong vapor activity by the box vapor assay. These oils were coriander, spike lavender, true lavender, lavandin, peppermint, tea tree, spearmint, helichrysum, cajeput, eucalyptus globulus, eucalyptus radiata, ravensara, and frankincense. High vapor concentrations of these oils in the sealed box may have contributed to the increase in the vapor activity, because they are known as top note oils, and because linalool, terpinen-4-ol, carvone, camphor, and 1,8-cineole which were the major components of these oils, behaved similarly, showing more enhanced vapor activity than that expected from the contact activity.

In contrast, cedarwood Virginia, patchouli, eupatorium, German chamomile, perilla (egoma), and galbanum showed moderate activity by the agar diffusion assay, but no activity by the box vapor assay. It was noted that many of these oils were very viscous liquids containing many sesquiterpenes and other components of low volatility. The major components of these oils were cedrol (32%) in cedarwood Virginia oil, patchoulol (42%) in patchouli oil, bisabolol oxide (34%) and α-bisabolol (12%) in German chamomile oil, and myristicin (59%) in perilla (egoma) oil. Eupatorium oil contained, as minor constituents, durenol (5%), selina-6-en-4-ol (3%), and an unidentified low-volatility component (16%), in addition to β-caryophyllene and dimethoxydurene. Galbanum oil contained three sesquiterpenols (12%) including guaiol, cubenol, and an

unidentified one as minor constituents, in addition to the major one, 3-carene. These major and minor sesquiterpenols may have contributed to the contact activity, but not to the vapor activity, because two sesquiterpenols, farnesol and cedrol, did not show any vapor activity, though they showed contact activity, as seen in Table 2. Other sesquiterpenols, such as α-bisabolol, nerolidol, and elemol, also showed contact activity, but no vapor activity (data not shown). The results suggested that oils containing sesquiterpenols as the active ingredient appeared to be inappropriate for vapor application. However, again, exceptional cases were noted. Sandalwood oil, containing α,β-santalol; myrrh oil, containing lindestrene, curzerene, and furanoeudesma-1,3-diene; and cedarwood Himalaya, containing α,β-himachalenes and atlantone as the major sesquiterpenes exhibited moderate vapor activity (25 μg/ml air), in contrast to other sesquiterpene- rich oils that were inactive in the vapor assay. The reason for this inconsistency is not known. Furthermore, cedarwood Himalaya was more potent in both vapor and contact activities than cedarwood Atlas, though both oils had the same sesquiterpenes as major components. The only difference observed was for the sesquiterpenols, which were himachalol and allo-himachalol (2%–3%) in cedarwood Himalaya, and atlantol (4%) in cedarwood Atlas. This difference was not enough to explain the biological difference between the two cedarwood oils. Regarding the monoterpene-rich oils, discrepanies between the vapor and contact activities of ravensara and frankincense oils remain to be resolved. As far as the monoterpene phenol, aldehyde, alcohol, and ketone oils were concerned, the major constituents appeared to be responsible for the vapor and contact activities. However, it was difficult to determine the bioactive principles of the ester, ether/oxide, and hydrocarbon oils, in which the major constituents had weak activity.

Suhr and Nielsen[21] reported that thyme, cinnamon, and clove oils, containing thymol and eugenol, had the best effect when applied to agar medium, and mustard and lemongrass, containing allyl isothiocyanate and citral, were most effective when added as volatiles. We observed no difference in activity between the agar diffusion assay and box vapor assay for thyme, clove, and lemongrass oils. The difference between our findings and theirs in regard to thyme and clove oils may be due to different methods of evaporation. They placed the essential oils themselves without dilution at the bottom of the jar, where the evaporation of the oil components was slow. In our study, essential oils were impregnated on filter paper to accelerate evaporation. Slow evaporation tended to decrease the vapor activity as compared with fast evaporation, as already reported.[22]

Essential oils such as oregano, thyme thymol, wild thyme, clove, cinnamon bark, and lemongrass that exhibit high vapor activity against a *T. mentagrophytes* may be useful as room disinfectants when patients with tinea pedis may drop the living pathogens on the floor. However, the oils containing an aldehyde component may not be useful for application to superficial infections, because the aldehyde functional group is readily oxidized by body fluids[23] and it has irritant properties on skin and mucous membranes.

Although, in this study, we examined anti-*Trichophyton* activity using only one strain – T. mentagrophytes TIMM2789—both *T. mentagrophytes* TIMM1189 and *T. rubrum* TIMM2659 showed susceptibility similar to that of *T. mentagrophytes* TIMM2789 when tested by agar diffusion and broth dilution assays using cinnamon bark, lemongrass, thyme thymol, perilla (shiso), true lavender, and tea tree oils (data not shown). The vapor activity of essential oils against clinical isolates of dermatophytes should be determined in future studies before vapor therapy with essential oils can be employed clinically.

It was concluded from the box vapor assay that the oils containing phenol or alcohol as the major constituent may be suitable for the treatment of tinea infection by vapor application under sealed conditions. However, in practice, perfect sealing might be difficult to achieve. Therefore, both potent vapor activity and potent contact activity may be required for anti-infectious therapy. Based on these criteria, the following candidate oils were selected: oregano, wild thyme, thyme thymol, clove, citronella, geranium "Bourbon", lindera, rosewood, thyme thujanol and thyme geraniol, lavender, and tea tree. A model experiment for the prevention of tinea pedis is underway in this laboratory, using these selected oils.

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