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Antifungal Activity and Inhibitory Modes of Volatile Vapours of Essential Oils

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Antifungal activities of volatile vapours of essential oils were investigated. Volatile vapours of test essential oils except Cedarwood and Pachouri showed inhibitory activity against test fungi. Volatile vapours of Birch essential oils exhibited fungistatic activity against dermatophytic filamentous fungi while others did fungicidal activity. Spores of dermatophytic filamentous fungi are more susceptible to volatile vapours of essential oils than mycelia.

KEYWORDS: Antifungal activity, Essential oil, Volatile vapour

Essential oils are natural organic substances which are produced in various glands and sacs of flowers, leaves, stems, woods, and roots of aromatic plants. The characteristic of essential oils is their volatile nature and they give strong aromatic odors. Therefore the essential oils of aromatic plants have been widely used in the production of cosmetics as oil supplements or perfumes. In recent the medicinal values of essential oils have attracted increasing interest. During the past decades a number of studies have been performed concerning the biological activities of the essential oils of aromatic plants (Baratt *et al.*, 1998; Beesley *et al.*, 1996; Hayashi *et al.*, 1995) The application of essential oil as an anti-microbial agent has also been studied. Apisariyakul *et al.* (1995) showed the antifungal activity of tumeric essential oil against human pathogenic fungi and yeasts. Filipowicz *et al.* (2003) studied the antibacterial and antifungal activity of juniper berry essential oil. Since fungal infection is more difficult to be treated owing to toxic side effect of antibiotics for human cell, the search for effective novel anti-fungal agents with minimum toxicity has always been challenging to reduce undesirable drug side effects and acquired resistances to pathogenic fungi.

Although there have been numerous reports on the antifungal activity of essential oils applied directly to fungus, the studies concerning the antifungal activity of volatile vapours of the essential oils were relatively limited (Jain and Agrawal, 1978, 2002) and few studies concerning inhibitory modes of essential oils have been reported.

In this study, we evaluated the antifungal activity of volatile vapours of commercially available essential oils against several fungi including *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporium canis* and *Candida albicans*, the casual agents of onymycosis as well as *Malassezzia furfur*, casual yeast of several pathological condition including pytiriasis versicolor, seborrhetic dermatitis and dandruff. We also investigated the inhibitory modes of volatile vapours of essential oils and compared the inhibitory exposure time of volatile vapours of essential oils between spore germination and mycelial growth.

All essential oils used were listed in Table 1. Essential oils were purchased from local cosmetic market in Seoul, Korea. *T. rubrum* strain 6345, *T. mentagrophytes* 2321, *M. canis* 4568, *M. furfur* 4325 *C. albicans* 7965 were obtained from KCTC (Korean Collection for Type Culture). Filamentous fungi and *C. albicans* were maintained on Saborad dextrose agar (SDA) at 26°C and 37°C, respectively until the study was performed. *M. furfur* were maintained on the surface of SDA covered with corn oils at 37°C. Volatile vapours of essential oils were tested against spore germination and mycelial growth of fungi.

Effects of essential oils on fungi were determined by observing the spore germination and mycelial growth in a saturated volatile aromatic atmosphere. Disposable Phytatray (Sigma, USA) with sterilized lid were used as chamber containing essential oil and test fungi. In order to perform spore germination test, cotton soaked in 100 *μl* of the test essential oil and a small petri dish (5 cm diameter) containing agar discs smeared with spores were placed in the Phytatray at dose level of 100 *μl*/800 *ml* air space. A set of phytatray was run as a control, in which no essential oil was applied. The Phytatrays containing fungi except *C. albicans* and *M. furfur* were incubated at 26°C for 8 days. The Phytatrays containing *C. albicans* and *M. furfur* were incubated at 37°C for 3 days. After incubation, spore germination was determined by microscopic observation. At least 50 spores was investigated for spore germination. Inhibition was determined by counting the number of germinating spores. Complete inhibition was determined if the number of germinating spores out of counted total spores was less than 10%. If the number of germinating

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Table 1. Antifungal activity of volatile vapours of essential oils against germination of spores of several fungi

Essensial oil	*Microsporium canis*	*Trichophyton rubrum*	*Trichophyton menta*	*Malissezia furfur*	*Candida albicans*
Tea tree	++++*	++++	++++	++++	++
Pachouli	–	–	–	–	–
Grapefruit	++++	++++	++++	++++	-
Myrtle	++++	++++	++++	++++	++++
Birch	++++	++++	++++	++++	++++
Cedarwood	–	–	–	–	–
YlangYlang	++++	++++	++++	++++	++++
Cajuput	++++	++++	++++	++++	++++
Cumin seeds	++++	++++	++++	++++	++++

* ++++: complete inhibition, ++: partial inhibition, –: no inhibition.

spores was more than 90%, no inhibition was considered. Other cases were considered as a partial inhibition.

In order to determine sporostatic or sporocidal activity of volatile vapour of essential oils, a loopful of non-germinated spores was taken from culture plate and was placed into fresh malt extract broth. Broths were incubated at 26°C for filamentous fungi or 37°C for *C. albicans* and *M. furfur* for 6 days. Fungi showing germinated spores were considered to be sporostatic whereas fungi with non-germinated spores would be sporocidal.

For mycelial growth test of *T. rubrum*, *T. mentagrophytes*, *M. canis*, and *A. niger*, 1-cm agar block (diameter) of test fungus was prepared from the margin of actively growing area of fungal colony. Agar block of test fungus was placed in Phytatray containing essential oil at dose level of 100 *μl*/800 *ml* air space. Control Phytatray without essential oil was prepared. Mycelial growth was assessed by measuring any increase of fungal colony diameter after incubation at 26°C for 6 days.

In order to determine fungistatic or fungicidal activity of volatile vapours of essential oils on mycelial growth, after removal of essential oils from Phytatray, plates were further incubated at 26°C for 6 days. Fungi resuming mycelial growth were considered to be fungistatic whereas fungi showing no more growth would be fungicidal.

In order to compare the inhibitory exposure time of volatile vapours of essential oils between mycelial growth and spore germination, *T. rubrum* and *M. canis* were used as test fungi. Essential oils such as Cajuput and Cumin seed showing potent antifungal activity was placed at dose level of 100 *μl*/800 *ml* in the Phytatray containing agar disc inoculated with spores or mycelial block of *T. rubrum* and *M. canis* respectively. Phytatrays were incubated at 26°C for 12, 24, 36 and 48 h. At each interval time, essential oil was removed from Phytatray. Phytatray was then further incubated for 5 days. After incubation spore germination or mycelial growth were investigated.

In order to compare the effectiveness of direct contact of essential oils with gaseous contact of volatile vapours, modified paper disc test was performed. Sterilized Whatman filter paper disc of 6 mm diameter was impregnated with each essential oils and placed over the center of surface of SDA plate seeded with fungal cells in petri dish. Petri dish was then incubated without covering of lid to minimize the effects of volatile vapours of essential oils. The cultures of *T. mentagrophytes*, *T. rubrum*, and *M. canis* were incubated for 6 days at 26°C. The cultures of *C. albicans* and *M. furfur* were incubated for 48 h at 37°C.

Our data demonstrated that volatile vapours of many essential oils was inhibitory against the germination of spores and mycelial growth of test fungi. Volatile vapours of most test essential oils exhibited inhibitory activity of mycelial growth and spore germination against all the test filamentous dermatophytic fungi such as *M. canis*, *T. rubrum*, and *T. mentagrophytes* while volatile vapours from essential oils of Cedarwood and Pachouri did not exhibit inhibitory activity (Tables 1 and 2). In inhibitory mode test, after removal of volatile vapour of Birch essential oil, test filamentous dermatophytic fungi resumed mycelial growth and spore germination. This result might indicate that volatile vapour of Birch essential oil possessed fungistatic activity of mycelial growth and spore germination rather than fungicidal activity at the dose level tested. Removal of other essential oils failed to resume mycelial growth and spore germination, considering to possess fungicidal activity. Susceptibility to volatile

Table 2. Antifungal activity of volatile vapours of essential oils on mycelial growth of dermatophytic filamentous fungi

Essensial oil	*Microsporium canis*	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
Tea tree	++++*	++++	++++
Pachouli	–	–	–
Grapefruit	++++	++++	++++
Myrtle	++++	++++	++++
Birch	++++	++++	++++
Cedarwood	–	–	–
YlangYlang	++++	++++	++++
Cajuput	++++	++++	++++
Cumin seeds	++++	++++	++++

*++++: inhibition, –: no inhibition.

Table 3. Inhibitory exposure time of volatile vapours of essential oils on *Microsporium canis*

Inhibition exposure time (h)	12		24		36		48	
Essential oil / Fungal growth	Cumin seed	Cajuput	Cumin seed	Cajuput	Cumin seed	Cajuput	Cumin seed	Cajuput
Spore germination	++*	++	++	++	++	++	++	++
Mycelial growth	–	–	++	++	++	++	++	++

*++: inhibition, –: no inhibition.

Table 4. Inhibitory exposure time of volatile vapours of essential oils on *Trychophyton rubrum*

Inhibition exposure time (h)	12		24		36		48	
Essential oil / Fungal growth	Cumin seed	Cajuput	Cumin seed	Cajuput	Cumin seed	Cajuput	Cumin seed	Cajuput
Spore germination	++*	++	++	++	++	++	++	++
Mycelial growth	–	–	–	++	++	++	++	++

*++: inhibition, –: no inhibition.

vapours of essential oils was different between spore germination and mycelial growth (Tables 3 and 4). In *T. rubrum*, 12 h exposure of volatile vapour of essential oil of cumin seed was enough to inhibit spore germination completely whereas 36 h exposure was required to inhibit mycelial growth completely. In *M. canis*, 12 h exposure of volatile vapour of essential oil of cumin seed was enough to inhibit spore germination completely while 24 h exposure was required to inhibit mycelial growth. These results suggested that spores of dermatophytic filamentous fungi were more susceptible to vapours of test essential oils than mycelia. In paper disc test, direct contact of all the test essential oils inhibited the growth of all the filamentous dermatophytic fungi (data is not shown). This result indicated that direct contact of fungal cells with essential oils might be more effective to inhibit fungal growth than volatile vapour.

In *C. albicans*, volatile vapours of essential oils of Myrtle, Birch, Cajuput, YlangYlang and Cumin seeds showed complete inhibitory activity (Table 1). Among essential oils, volatile vapours of Cumin seed and Cajuput essential oils exhibited fungicidal activity since malt extract broth inoculated with a loopful contents from inhibition zone showed no turbidity after 48 h incubation at 37°C. In *M. furfur*, all the volatile vapours of essential oils except Pachouri and Cedarwood completely inhibited yeast growth (Table 1). Among inhibitory essential oils, Cumin seeds and Cajuput showed fungicidal activity since malt extract broth inoculated with a loopful content from inhibition zone did not show turibidity after incubation. In paper disc test, all the test essential oils with the exception of Cedarwood exhibited inhibitory activity against *C. albicans* and *M. furfur*.

In this study, volatile vapours of essential oils such as Cumin seed and Cajuput exhibited potent antifungal activity with fungicidal inhibition mode. Zaika (1988) indicated that the volatile vapours of essential oils could be disadvantageous since the inhibitory agents of volatile vapours could be vanished by evaporation following prolonged exposure time, allowing the fungus to resume growth. However, although direct contact of essential oils with fungal cells was more effective to inhibit fungal growth and spore germination, volatile vapours of some essential oils possessed fungicidal activity at high dose level, preventing to resume growth after removal of essential oils. Therefore antifungal activity of volatile vapours of essential oils could be widely applicable in the variety of external dermal fungal infection treatment and prevention.

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