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Antifungal Activity of Clove Essential Oil and its Volatile Vapour Against Dermatophytic Fungi

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Antifungal activities of clove essential oil and its volatile vapour against dermatophytic fungi including *Candida albicans*, *Epidermophyton floccosum*. *Microsporum audouinii*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum* were investigated. Both clove essential oil and its volatile vapour strongly inhibit spore germination and mycelial growth of the dermatophytic fungi tested. The volatile vapour of clove essential oil showed fungistatic activity whereas direct application of clove essential oil showed fungicidal activity.

KEYWORDS: Antifungal activity, Clove oil, Dermatophytic fungi, Volatile vapour

Essential oils are becoming increasingly popular to be used for a wide variety of purposes, including aromatherapeutics and alternative natural medicines. During the last few years, due to the increasing development of drug resistance to antifungal agents in human dermatophytic fungi, demand for searching novel antifungal agents is being increased. The plant essential oils as natural substances could represent a potential source of new antifungal agent. During the past years, a number of studies have been carried out concerning the application of essential oils as antimicrobial agents (Barrtta *et al.*, 1998). In most cases, reports on the antifungal activity of essential oils exposed directly to fungus were published. However, only few studies concerning the antifungal activity of volatile vapour of the essential oils have been performed.

Clove has been used medicinally in the field of oriental herbal medicine and as a culinary spices. Plant's flower bud is used for both flavoring and from which the essential oil is extracted. The characteristics of its essential oil is volatile nature and strong aromatic odors. Therefore, the essential oil of clove has been widely used in aromatherapy and cosmetics. It has been also used as a natural anesthetic in dentistry. In a review article, Kalemba and Kunicks (2003) reported that direct application of essential oil of clove was effective against *Aspergillus parasitica*, *Candida albicans*, and *Cryptococcus neoformans*. However, report on the antifungal activity of volatile vapour of clove essential oil was limited. The aim of this study is to investigate the clove essential oil and its volatile vapour as an antifungal agent against dermatophytic fungi.

Clove essential oil was purchased from cosmetic market in Korea. *C. albicans* KCTC 7965, *Epidermophyton floccosum* KCTC 6586, *Microsporum audouinii*, *Trichophyton mentagrophytes* KCTC 6316, and *Trichophyton rubrum* KCTC 6345 were obtained from Korean Collection for Type Culture (KCTC). Strains were maintained on Sabouraud dextrose agar (SDA) at 26°C. Effect of volatile vapour of clove essential oil on fungi were determined by Phytatray chamber assay. Disposable Phytatray (Sigma, USA) with sterilized lid was used as a chamber containing essential oil and fungus. Inhibitory activities of clove essential oil were investigated on cell growth of *C. albicans* and spore germination of *T. mentagrophytes* and *T. rubrum*. SDA plates inoculated with cells of *C. albicans* and spores of *T. mentagrophytes* and *T. rubrum* were prepared. In order to collect spore suspension from *T. mantagrophytes* and *T. runbrum*, 10 day-old culture was flooded with sterile distilled water and was gently agitated to recover spores. An 800 *μl* of clove oil contained in small vial and SDA plate inoculated with yeast cells or spores were placed in the 800 *ml* volume of Phytatray. A set of phytatray was run as a control, in which no essential oil was applied. The Phytatrays were incubated at 26°C for 7 days. After incubation, spore germination was determined by microscopic observation. Inhibition was determined by counting the number of germinating spores out of counted total spores was less than 10% in microscopic field.

In order to demonstrate the sporostatic or sporocidal activity of volatile vapour of clove essential oils, spores were harvested from culture plate which shows the inhibition of germination and were placed into fresh malt extract broth. Culture broths were incubated at 26°C for 5 days. Fungi showing spores germinated were considered to be sporostatic whereas fungi with non-germinated spores would be sporocidal.

For mycelial growth test, 5 mm agar blocks (diameter)

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of *E. floccusom* and *M. audouinii* were taken from the margin of actively growing area of fungal colonies. Agar blocks were placed in Phytatray with 800 *ml* air space containing essential oil at dose level of 1 *μl*/1 *ml* air space. Control Phytatray without essential oil was prepared. Mycelial growth was determined by observing the further growth of fungal colony after incubation at 26°C for 7 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.

In order to estimate the effect of direct exposure of essential oils on test fungi, broth dilution assay was performed. For *T. mentagrophytes*, and *T. rubrum*, spore germinations were investigated. Spore suspensions were prepared as above and were adjusted with distilled water to 1×10^7 spores/*ml* using hematocytometer. Cells of *C. albicans* were also adjusted to 1×10^7 spores/*ml*.

For the broth dilution assay, malt extract broth was dispensed into each well of 96 well plate. Then each well was inoculated with 10 *μl* of spore suspension or cells prepared as above. For *E. floccosum* and *M. audouinii*, mycelial growth was investigated. Each well was inoculated with 5 mm diameter agar block taken from the margin of actively growing area of fungal colony. For all broth microdilution assay, clove oils at various concentration were added to each well. Tween 80 was added to each well at 0.5%. Plates were incubated at 26°C for 5 days. In controls, sterile water and Tween 80 at concentration equivalent to that in test solution were added to each well instead of clove oil. Hyphae developed from the spores or further hyphal growth present were observed. The minimum inhibitory concentration (MIC) value was determined as the lowest concentration at which no growth of fungal cells were observed. In order to investigate whether clove oil shows fungicidal or fungistatic activity for fungi, spores, cells and mycelia were collected from culture broth showing MIC, and washed in the distilled water to remove the remaining test solutions. Subcultures were prepared in fresh malt extract broth without supplement of test solution. After 3 days incubation at 26°C, microscopic observations were carried out to investigate fungal cell growth.

Our results showed that the volatile vapour of clove essential oil was strongly active to all of the fungi tested except *C. albicans*. Spore germinations of *T. mentagrophytes* and *T. rubrum* were completely inhibited by the volatile vapour of clove essential oil (Fig. 1). The volatile vapour of clove essential oil also strongly inhibited the mycelial growth of *E. floccusom* and *M. audiouinii* (Fig. 2). After the removal of clove essential oil from Phytatray, resumption of spore germination or mycelial growth were investigated after 24 h incubation, representing fungistatic activity of the volatile vapour of clove essential oil after 24 h incubation. However, the volatile vapour of clove

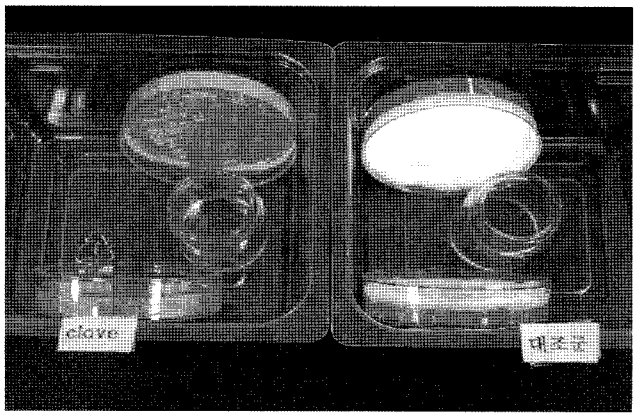
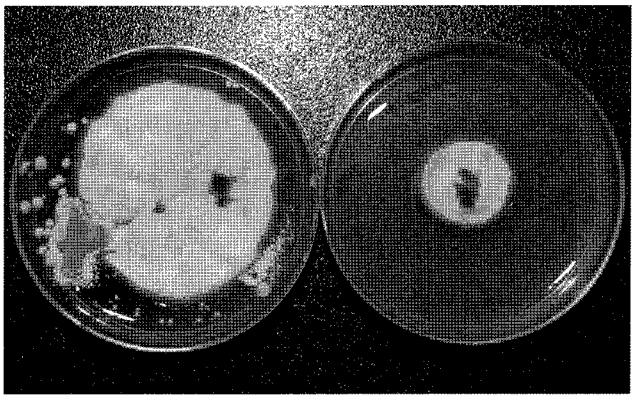


Fig. 1. Phytatray chamber assay of clove essential oil. Left Phytatray represents the complete inhibition of spore germination of *T. mentagrophtes* and *T. rubrum* with clove oil. Right Phytatray represents the full growth of *T. mentagrophtes* and *T. rubrum* without clove oil.

(A)



(B)

Fig. 2. Mycelial growth inhibition of clove essential oil against. *E. floccosum* (A) and *M. audouinii* (B) in Phytatray chamber assay (right plate: clove oil treatment. left plate: control).

Table 1. MIC (% v/v) of clove essential oil against fungi tested

Fungi	MIC (% v/v)
C. albicans	2.5
E. floccusom	5
M. audiounii	2.5
T. mentagrophytes	1
T. rubrum	1

essential oil showed a very weak activity against *C. albicans*. Although the volatile vapour of clove essential oil could reduce the growth rate of *C. albicans* cells, full growth of yeast cells was observed after 48 h. This result was in disagreement with that of Briozzo *et al.* (1989). However, their results were based on the direct exposure of clove essential oil in a concentrated sugar solution to *C. albicans*.

In our studies, direct application of clove essential oil in broth dilution assay showed potent antifungal activities against the test fungi. MICs of clove oil were 1% for *T. mentagrophytes* and *T. rubrum*. Mycelial growth of *E. floccusom* and *M. audiouinii* in broth assay was completely inhibited at 5% and 2.5%, respectively (Table 1). Essential oil of clove also strongly inhibited the growth of *C. albicans* at MIC of 2.5%. No further growth of mycelia or resumption of spore germination were observed, representing fungicidal activity of clove essential oil in broth dilution assay.

In our studies, volatile vapour of clove essential oil showed fungistatic activity on solid medium, whereas the oil demonstrated fungicidal activity in broth medium. Therefore we suspect that clove essential oil and its volatile vapour may have different mode of antifungal activity against test fungi. Jain and Agrawal (2002) demonstrated that fungistatic activity of volatile vapour of several essential oils against fungi. To our knowledge, no other author reported antifungal activity of volatile vapour of clove oil. Although the exact mechanism of fungal growth inhibition by volatile vapour of essential oils was not revealed, it was suggested that the volatile compounds of essential oils influence on a variety of cell metabolism (Fries *et al.*, 1973). Further research will be needed to investigate the mechanism of antifungal activity of clove essential oil.

In present study, volatile vapour of clove essential oil demonstrated to be an effective antifungal agent against several human pathogenic fungi. Therefore, using volatile vapour of essential oils as an antifungal agent may provide a wide and useful application for several aspects including aromatherapy, lense preservation, and the treatment of Athelet's foot disease.

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