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Effect of Combined Cinnamon and Clove Oil Against Major Moulds Identified from Rubberwood (*Hevea brasiliensis*)

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ABSTRACT

Antimicrobial activities of pure and combined cinnamon and clove oils in a ratio of 1:1, 1:3, 1:5, 1:7, 3:1, 5:1 and 7:1 against major mould found on rubberwood (*Aspergillus niger* WU001, *Penicillium chrysogenum* WU002, *Penicillium* sp. WU003) were investigated using a broth dilution method at a concentration of 10 - 100 μL/mL. The higher ratios of cinnamon and clove oils (3:1, 5:1 and 7:1) were stronger inhibitors than the lower ratios of cinnamon and clove oils (1:1, 1:3, 1:5 and 1:7) and the pure cinnamon or clove oil. The minimum inhibitory concentration (MIC) of the combined cinnamon and clove oils at the ratio 5:1 was determined to be 50 μL/mL for all moulds. Antifungal activity of the combined cinnamon and clove oils (5:1) at a concentration of 50 μL/mL was further examined on rubberwood specimens under storage conditions of 30 °C and 100 %RH. It was found that all moulds on rubberwood specimens were completely inhibited for at least 12 weeks under the storage conditions examined.

Keywords: Rubberwood, cinnamon oil, clove oil, antifungal

INTRODUCTION

Rubberwood (*Hevea brasiliensis*) has been recognized as an environmentally friendly wood material for a number of years. Mould such as brown rot (e.g. *Coniophora puteana*) and white rot (e.g. *Trametes versicolor*) are observed on the wood [1]. Moreover, *Aspergillus niger* and *Penicillium chrysogenum* have also reported as wood mould [2]. To preserve rubberwood from fungal attack, chemicals such as boron compounds are commonly impregnated into rubberwood [3]. This, however, has narrowed down the utilization of rubberwood mainly to the furniture industries. To extend rubberwood utilization to other applications where health is of greater concern such as food related materials and children's play toys, the application of harmless natural preservatives extracted from herbs or plants is, therefore, an interesting alternative.

Cinnamon oil and clove oil are natural preservative substances that are not harmful. There have been a number of reports indicating that some of the substances in cinnamon oil and clove oil inhibit the growth of moulds, yeasts and bacteria. Soliman and Badeaa [4] found that ≤ 500 ppm of cinnamon oil could inhibit *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme* on a potato dextrose agar (PDA) medium. August [5] reported that high concentrations of cinnamon and clove oil inhibited the asexual spores of mould. Both cinnamon oil and clove oil at a 2 % level in PDA completely inhibited the growth of seven mycotoxigenic moulds: *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium* sp. M46, *P. roqueforti, P. patulum* and *P. citrinum* for various times up to 21 days [6] and could also inhibit the growth of yeasts [7].

The objective of the research was to study the inhibitory effects of cinnamon and clove oils, added singly and in various combinations, on the growth of mould, commonly found on rubberwood.

MATERIALS AND METHODS

Cinnamon oil and Clove oil

Cinnamon oil (containing 70 - 74 % cinnamaldehyde and 5 - 9 % eugenol) and clove oil (containing 75 - 80 % eugenol as the major ingredient) were purchased from AromaSense Co., LTD. Auckland, New Zealand.

Cultures

Three strains of moulds (*A. niger* WU001, *P. chrysogenum* WU002, and *Penicillium* sp. WU003) were identified from rubberwood surfaces. Codes refer to strains held in the culture collection of the Wood Science and Engineering Research Unit, Center

for Scientific and Technological Equipment, Walailak University, Nakhon Si Thammarat, Thailand.

Preparation of inoculum

Spores were obtained from mycelium grown on a Malt Extract Agar (MEA; Merck Ltd, Thailand) medium at 30 °C for 14 days and were collected by flooding the surface of the plates with ~ 5 ml sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1 % v/v). After counting the spores, the solution was standardized. Mould growth was observed after inoculation of 10^7 spores at the center of the plates (10 µl of a standardized suspension 10^7 spores/ml).

Minimal inhibitory concentration (MIC)

The broth dilution method as described by Rasooli and Abyaneh [8] was employed. 10 - 100 µL/mL of cinnamon and clove oil were incorporated singly or as a mixture of oils in ratios of 1:1, 1:3, 1:5, 1:7, 3:1, 5:1 and 7:1. These mixtures were added to 5 ml of a yeast extract sucrose broth (YES). The control was handled in the same way, but vegetable oil was added to the YES broth. Six replicates were prepared for each treatment. The tubes were then incubated at 30 °C for 3 days on an incubator shaker (Gallenkamp, Loughborough, England) to evenly disperse the oil throughout the broth in the tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as the Minimum Inhibitory Concentration (MIC).

Moulds test on rubberwood

Rubberwood specimens (7 mm × 20 mm cross section by 7 cm long) were prepared from freshly cut rubberwood lumber obtained from a plantation in Nakhon Si Thammarat, Thailand. The average moisture content of the rubberwood before testing was 49±2 % (n = 10). Rubberwood specimens (n = 6) were dipped in 70 % ethanol for 15 s and put into the sterile plate (90 mm) before evaporating. Then, the treated wood was immersed in 50 samples with a combination of cinnamon and clove oil at a ratio of 5:1. The treated wood was held in a covered container overnight according to the ASTM standard test method D4445-91 [9]. An untreated specimen was dipped in 70 % ethanol and dipped in vegetable oil to serve as a control for the test. After spraying with 1 mL of the mixed mould-spore inoculum (10^7 spores /ml), the specimens were kept in polyethylene bags to prevent drying, and incubated at 30 °C with 100 % relative humidity (%RH) for 12 weeks. Following incubation, specimens were investigated and individually rated for mould growth on a scale of 0 to 5, with 0 denoting clean specimens and 5 representing heavy mould growth (0 = clean, 1 = 20 %, 2 = 40 %, 3 = 60 %, 4 = 80 %, 5 = 100 % of mould growth).

Statistical analyses

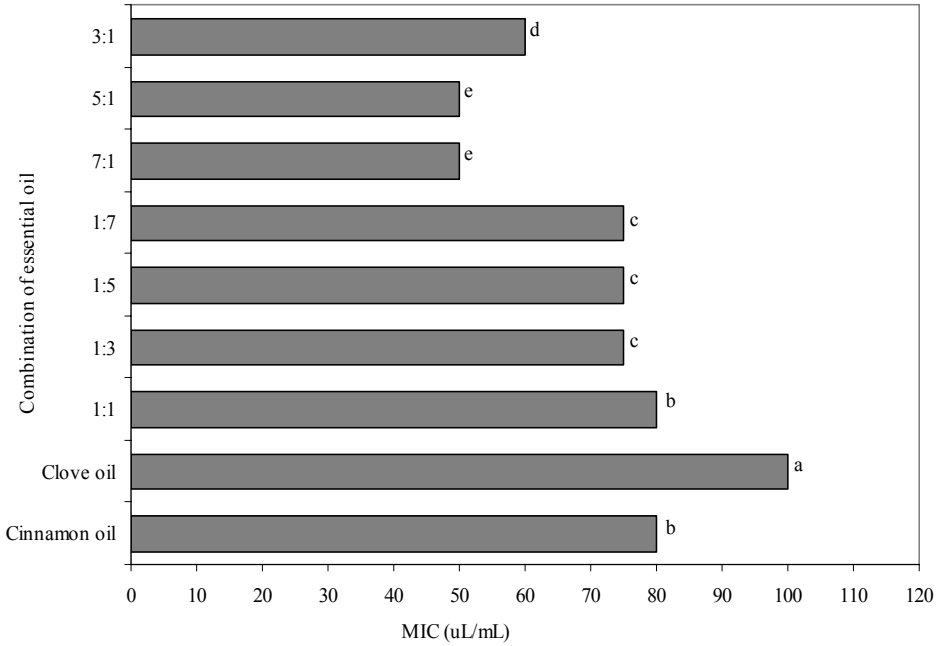
All variables were tested for normality applying the Komogorov-Smirnov test and homogeneity of variances was assessed using Levene's test. Data transformation was done, where necessary. All results were expressed as mean ± SD ($n = 6$). The data was statistically treated by one-way ANOVA and Duncan's post hoc test with $P < 0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

Inhibition of mould by cinnamon and clove oils

The inhibition of mould by cinnamon and clove oils were determined *in vitro* by the dilution method. Results are shown in **Figure 1**. The MIC values of all conditions examined were coincident. It is evident that all essential oils exhibited fungistatic activities on the test moulds. Cinnamon oil showed inhibition of all moulds at 80 - 85 µL/mL and clove oil showed fungistatic activities at 85 - 100 µL/mL against all moulds. Combination of cinnamon oil and clove oil at ratio 1:1, 1:3, 1:5 and 1:7 (75 - 80 µL/mL) and at ratio 3:1, 5:1 and 7:1 (50 - 60 µL/mL) were required to inhibit growth of all moulds. The result showed clearly that increasing the ratios of cinnamon oil (3:1, 5:1 and 7:1) significantly reduced the growth rate of all moulds compared with lower ratios of cinnamon oils (1:1, 1:3, 1:5, and 1:7). Growth of moulds was clearly retarded at a higher cinnamon oil concentration. Moreover, the combination of cinnamon and clove oil at ratios 5:1 and 7:1 showed stronger inhibition than clove oil and cinnamon oil alone. These results are in good agreement with those reported by Matan and co-workers [10] for intermediate moisture food products.

Cinnamon oil was reported to consist of many components such as cinnamaldehyde, eugenol, and linalool [11,12]. The major component of clove oil is eugenol with small amounts of cariophyllene and humulene [13]. Components of cinnamon and clove oils were reported to be capable of inhibiting growth of insects [14]. The results from this work clearly indicate that the synergic reaction between some components of cinnamon oil and clove oil takes place and affects the growth of mould at ratios of cinnamon oil to clove oil higher than 5:1. Identification of the active components in the combined cinnamon and clove oils warrants further study.


3:1
5:1
7:1
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1:1
Clove oil
Cinnamon oil
d
e
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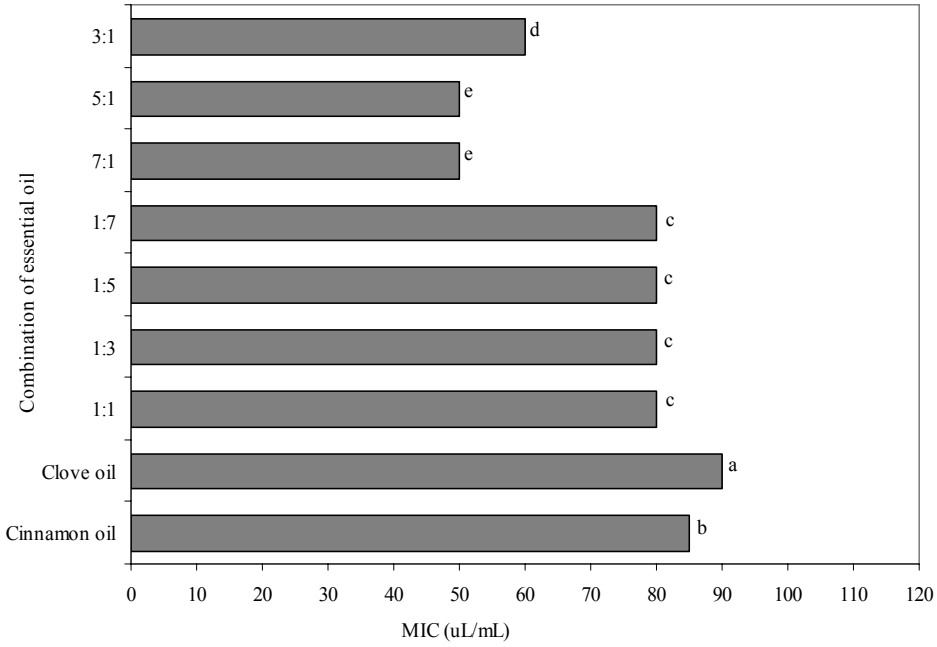
Combination of essential oil
0 10 20 30 40 50 60 70 80 90 100 110 120
MIC (uL/mL)

(a)


3:1
5:1
7:1
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1:3
1:1
Clove oil
Cinnamon oil
d
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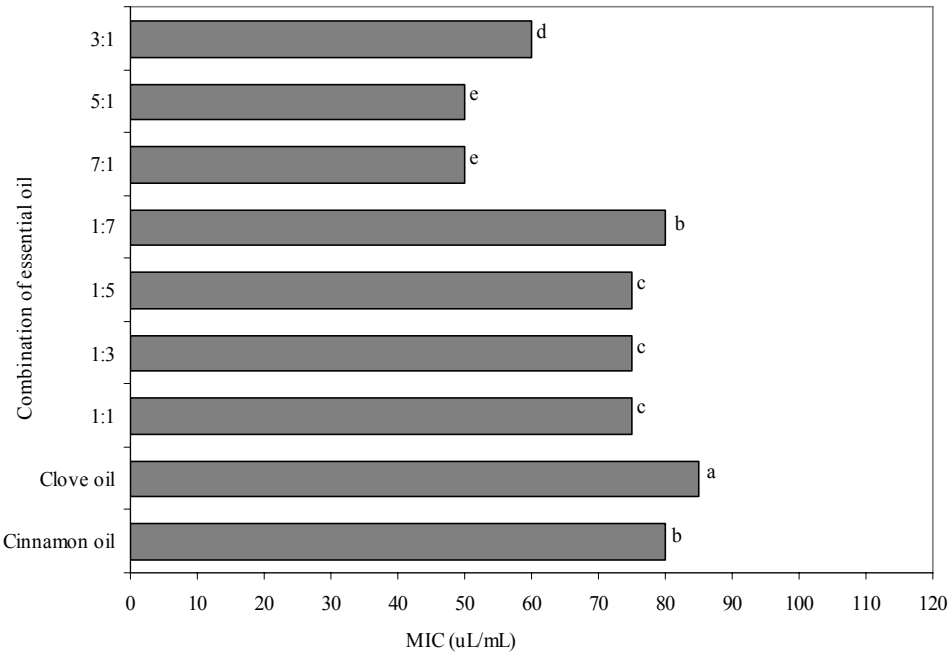
Combination of essential oil
0 10 20 30 40 50 60 70 80 90 100 110 120
MIC (uL/mL)

(b)



(c)

Figure1 Minimum inhibitory concentration (MIC) of clove oil and cinnamon oil alone and combinations of cinnamon and clove oil (1:1, 1:3, 1:5, 1:7, 7:1, 5:1 3:1) at 10 - 100 µL/mL against (a) *Aspergillus niger* WU001 (b) *Penicillium chrysogenum* WU002 (c) *Penicillium* sp. WU003.

[a-e] Significant $P < 0.05$.

Moulds test on rubberwood

Rubberwood specimens were inoculated with mould according to the ASTM standard test method D4445-91 (1998a). The results are presented in **Table 1**. After 12 weeks, treated rubberwood specimens {50 µL/mL of cinnamon and clove oils (5:1)} showed a strong resistance to mould coverage (no growth) whereas the controls reached a value of 100 % mould growth under the conditions employed (**Figure 2**). The mycelium of mould was observed on the surface of the control specimens after 4 weeks. Mixtures of cinnamon and clove oils were effective for inhibiting growth of mould on rubberwood. The leaching characteristics of essential oil components from rubberwood under various conditions is another key area that should be explored in the future.

Table 1 Mould growth on rubberwood specimens treated with cinnamon and clove oils (5:1) at concentrations of 50 µg/mL under storage conditions of 30 °C at 100 %RH.

Fungi	Treatment	Average* mould growth rating at various times (weeks)			
		3	6	9	12
Aspergillus niger WU001	Control	0.0	5.0	5.0	5.0
	50 µg/mL	0.0	0.0	0.0	0.0
Penicillium chrysogenum WU002	Control	0.0	5.0	5.0	5.0
	50 µg/mL	0.0	0.0	0.0	0.0
Penicillium sp. WU003	Control	0.0	5.0	5.0	5.0
	50 µg/mL	0.0	0.0	0.0	0.0

* $n = 6$

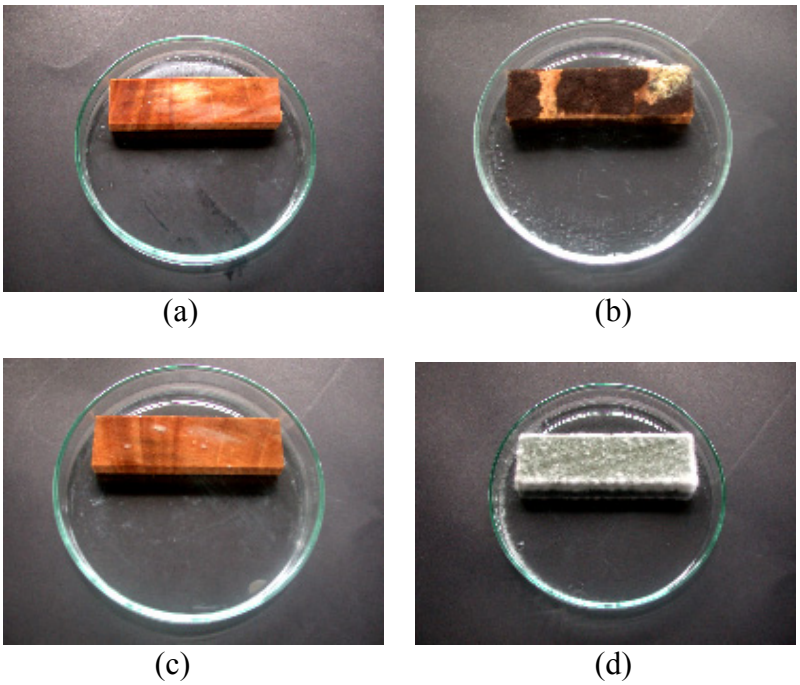


Figure 2 Growth of *Aspergillus niger* WU001 on rubberwood with 50 µL/mL of cinnamon oil and clove oil at ratio 5:1 after 12 weeks at 30 °C, 100 %RH (a), *A. niger* WU001 on rubberwood with vegetable oil (b), *Penicillium* sp. WU003 on rubberwood with 50 µL/mL of cinnamon oil and clove oil at ratio 5:1 after 12 weeks at 30 °C, 100 %RH (c), and *Penicillium* sp. WU003 on rubberwood with vegetable oil (d).

CONCLUSIONS

The following conclusions can be drawn from this work. The combination of cinnamon and clove oil showed good potential to inhibit the growth of rubberwood moulds i.e. *A. niger*, *P. chrysogenum,* and *Penicillium* sp. The optimum ratio of cinnamon and clove oils tested on broth was 5:1 with an MIC of 50 µL/mL for all mould. The mixture of cinnamon and clove oil at a ratio of 5:1 at a concentration of 50µL/mL was found to completely inhibit three tested moulds on rubberwood for at least 12 weeks at storage conditions of 30 °C and 100 %RH.

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บทคัดย่อ

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ผลของน้ำมันหอมระเหยจากอบเชยและกานพลูในการยับยั้งเชื้อราที่แยกมาจากผิวหน้าของไม้ยางพารา

งานวิจัยทำการศึกษาหาความเข้มข้นต่ำสุดตั้งแต่ 10 ถึง 100 ไมโครลิตร/มิลลิลิตรของน้ำมันหอมระเหยจากอบเชย กานพลู และส่วนผสมของน้ำมันหอมระเหยจากอบเชยและกานพลูในอัตราส่วน 1:1 1:3 1:5 1:7 3:1 5:1 และ 7:1 ที่ผสมในอาหารเหลวในการยับยั้งการเจริญของเชื้อรา *Aspergillus niger* เชื้อรา *Penicillium chrysogenum* และ เชื้อรา *Penicillium* sp. ซึ่งแยกได้จากผิวหน้าของไม้ยางพารา ผลการทดลองพบว่าน้ำมันหอมระเหยจากอบเชยและกานพลูที่อัตราส่วน 3:1 5:1 และ 7:1 มีประสิทธิภาพในการยับยั้งการเจริญของเชื้อราทั้ง 3 ชนิดมากที่สุดเมื่อเปรียบเทียบกับน้ำมันหอมระเหยในอัตราส่วน 1:1 1:3 1:5 และ 1:7 และน้ำมันหอมระเหยเพียงชนิดเดียว และพบว่าที่อัตราส่วนของน้ำมันหอมระเหยจากอบเชยและกานพลู 5:1 ใช้ปริมาณความเข้มข้นต่ำสุดในการยับยั้งการเจริญของเชื้อราทั้ง 3 ชนิดโดยใช้ปริมาณเพียง 50 ไมโครลิตร/มิลลิลิตร และเมื่อนำเอาน้ำมันหอมระเหยจากอบเชยและกานพลูในอัตราส่วน 5:1 ความเข้มข้น 50 ไมโครลิตร/มิลลิลิตร มาทดสอบความต้านทานเชื้อราบนไม้ยางพารา พบว่าสามารถยับยั้งการเจริญของเชื้อราบนไม้ยางพาราได้อย่างน้อย 12 สัปดาห์ที่อุณหภูมิ 30 องศาเซลเซียส ความชื้นสัมพัทธ์ 100 เปอร์เซ็นต์

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