Original article

Antifungal activity of Vietnamese essential oils against dermatophytes fungi

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Abstract

Background: Dermatophytosis is a prevalent disease with high incidence reported in tropical countries such as Vietnam. Currently, there are not many available types of antifungal drugs for dermatophytosis, and prolonged treatment is usually required. Besides, drug resistance in dermatophytes is a major concern in dermatology practice, especially that of *Trichophyton indotineae*. **Objectives:** Evaluating the antifungal activity of some local natural essential oils from Vietnam, mainly from Hue City, against species of dermatophytes belonging to genera *Trichophyton, Microsporum,* and *Nannizzia.* **Materials and Methods:** Essential oils including *Melaleuca cajuputi, Citrus grandis var. Thanh trà, Cinnamon, Cymbopogon citratus, and Mentha arvensis L* were tested. The essential oils were diluted with RPMI medium at various concentrations and then cultured with 16 dermatophyte strains of *Microsporum canis, Nannizzia incurvata, Trichophyton indotineae, Trichophyton interdigitale,* and *Trichophyton rubrum* to determine Minimum Inhibitory Concentrations (MICs) of these essential oils after three, five and seven days following EUCAST guidelines version 11.0. **Results:** All tested essential oils exhibited antifungal activity against dermatophyte species. The MIC of *Cymbopogon citratus* (0.05 - 0.08%) was significantly lower than the values of *Laurus cinnamomum (cinnamon)* (0.1 - 0.18%), *Mentha arvensis L.* (0.19 - 0.5%)*, Melaleuca cajuputi* (0.43 - 0.99%)*,* and *Citrus grandis var. Thanh trà* (0.52 - 1.09%) ($p < 0.001$). *Laurus cinnamomum (cinnamon)* oil also proved better inhibitory ability than the other three oils; *Mentha arvensis L.* oil demonstrated lower MIC than *Citrus grandis* and *Melaleuca cajuputi* oil ($p < 0.01$). Data analysis showed that *Cymbopogon citratus*, *Cinnamon,* and *Mentha arvensis L.* oils maintained stable MIC values over 3 and 5 days, but increased MICs on the 7th day, meanwhile *Citrus grandis* oil MIC values, despite being high, remained unchanged from the 5th to 7th day. Regarding *T. indotineae*, the strains were inhibited by *Mentha arvensis L., Cymbopogon citratus,* and *Cinnamomum* oils, all of which maintained MIC values within 0.2% over 7 days of incubation. **Conclusion:** This is the first study in Vietnam to evaluate antifungal activity of essential oils against dermatophyte strains. All essential oils demonstrated antifungal activity against dermatophyte species. *Cymbopogon citratus*, *Mentha arvensis L, Cinnamon*, *Citrus grandis var. Thanh trà* are potential candidates for further research.

Keywords*: dermatophytes, natural essential oils, antifungal activity.*

1. INTRODUCTION

Dermatophytes are keratinophilic fungi belonging to the genera *Trichophyton, Microsporum, Epidermophyton, Nannizia, Arthroderma, Lopophyton, Ctenomyces, Guarromyces,* and *Paraphyton* [1]. Dermatophytosis is a common disease, estimated to affect 20 - 25% of the global population [1]. Its high prevalence is reported in countries with hot and humid climates, including Vietnam [2, 3]. Factors facilitating dermatophyte infections include age, gender, season, socioeconomic conditions, geographic location, etc [1].

Dermatophytosis, though not a critical condition, can cause very unpleasant symptoms such as itching, skin lesions that affect appearance as well as quality of life, increase the risk of opportunistic infections if left untreated. Topical treatments are preferred over systemic ones due to potential side effects of antifungal medications [4]. However, treatment can be prolonged, and topical treatments may still carry a risk of side effects in patients [4]. Additionally, antifungal resistance, especially to terbinafine, a drug commonly used for treatment, has been reported recently, notedly with the *Trichophyton indotineae* species [5, 6, 7].

Accordingly, researching new antifungal agents, particularly those derived from natural sources, is essential for enhancing treatment efficacy and

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minimizing side effects. Several studies have reported the antibacterial and antifungal properties of natural essential oils [8, 9, 10, 11]. Besides, investigating the antifungal activity of essential oils from countries with many highly regarded medicinal plants like Vietnam is crucial to harness the potential of locally sourced plants. Hue City, a region renowned for high-quality annual production of essential oils extracted from local plants that are not only consumed domestically but also exported worldwide [12, 13]. Therefore, we are conducting a study titled "**Antifungal activity of essential oils against dermatophyte fungi**" aimed at assessing the inhibitory potential of various essential oils from Vietnam, particularly Hue City, against fungi in the *Trichophyton*, *Microsporum*, *Nannizzia* genera.

2. MATERIALS AND METHOD

Study design and participants

This study was conducted as a laboratory experiment from 9/2023 to 3/2024.

Sixteen dermatophyte strains belonging to the three genera *Trichophyton, Microsporum*, and *Nannizzia* were collected from patient samples (skin, hair and nail) between 2020 and 2023. These strains were identified through gene sequencing and stored by the Department of Parasitology, Hue University of Medicine and Pharmacy, including the following species: *Microsporum canis* (n = 3), *Nannizzia incurvata* (n = 3), *Trichophyton indotineae* (n = 3), *Trichophyton interdigitale* (n = 3), *Trichophyton rubrum* (n = 4). 100% of the tested strains were resistant to fluconazole, except for *T. rubrum*, which showed a resistance rate of 50%. All the fungal species strains were sensitive to itraconazole, voriconazole, and terbinafine, except for *T. indotineae*, which had a terbinafine resistance rate of 66.7% (2/3 strains tested).

Four commercial natural essential oils from Lien Minh Xanh Company (Hue City, Vietnam) contained extracts of *Cymbopogon citratus* (lemongrass), *Mentha arvensis L* (peppermint), *Melaleuca cajuputi* (cajeput), and *Citrus grandis var. Thanh trà* (pomelo). Also, *Laurus cinnamomum* (cinnamon) essential oil purchased from Sumo Nhat Viet Company (Hanoi City, Vietnam), along with other materials such as DMSO solvent (HiMedia, India), RPMI medium (HiMedia, India), potato dextrose agar (HiMedia, India), Sabouraud - Chloramphenicol - Cycloheximide medium (HiMedia, India), as well as 96-well microplates were used.

Procedure to evaluate antifungal activity of the essential oils

We determined the minimum inhibitory concentration (MIC) of essential oils that inhibit the growth of dermatophyte strains by modifying the EUCAST version 11.0 protocol [14]. Essential oil concentrations ranging from 0.005% to 1.25% were tested using the microdilution method. The MIC was defined as the lowest concentration (%) of essential oil that inhibited more than 50% of fungal growth.

Preparation of fungi: The stored fungal strains were cultured on Sabouraud - Chloramphenicol - Cycloheximide medium for 7 days, followed by subculturing onto potato dextrose agar for 5 - 7 days. Spores were then collected by overlaying 2 mL of RPMI medium and 1 drop of Tween 20 onto the colony surface. The spore suspension was collected and adjusted to 0.5 McFarland. This suspension was supplemented with chloramphenicol at a concentration of 50 mg/L and cycloheximide at 300 mg/L (this antifungal does not inhibit the growth of dermatophytes) [14]. The spore suspension was then diluted with RPMI medium at a ratio of 1:10.

Preparation of essential oils: 2.5% essential oil solution was prepared by mixing 2.5% stock essential oil + 10% DMSO + RPMI medium to complete the solvent volume containing 2.5% essential oil.

Procedure: Adding the fungal suspension, RPMI medium, 2.5% essential oil solution to each well in corresponding volumes to achieve essential oil concentrations from well 1 to well 9, ranging from 1.25% to 0.005%. Well 10 served as negative control (only contained RPMI medium), well 11 as positive control (RPMI medium + fungal suspension), and wells in the 12th row as DMSO controls that contain RPMI and DMSO with concentrations ranging from 0.04% to 5%, ensuring that the solvent at these concentrations did not affect fungal growth.

Result reading by visual observation: Observe turbidity changes compared to the positive control wells, verify the results using a spectrophotometer at 530nm wavelength, and confirm by using an inverted microscope [14].

Experimental quality control: After 3, 5, and 7 days of culture, the negative controls showed that the medium remained clear, indicating that the procedure ensured sterility; meanwhile in the positive controls, the fungi grew well, and DMSO control wells also showed fungi growth, proving that DMSO solvent at concentrations ranging from 0.015% to 4% did not affect the test results.

Data analysis

The data were processed using medical statistical methods by the SPSS 20.0 software. Qualitative variables are described by mean, median (mode),

and standard deviation (SD: Standard Deviation). The Chi-square test was used to compare two means, with the statistical significance set at $p < 0.05$.

3. RESULTS

In this study, the results in the control wells were appropriate as mentioned above, particularly noting that the fungi in the DMSO wells still grew, ensuring that the solvent had no antifungal activity and did not affect the results.

3.1. Minimum Inhibitory Concentration (MIC) of each essential oil against tested dermatophytes

All tested essential oils were able to inhibit the growth of dermatophyte fungi at all time points studied. (**Table 1**)

Table 1. Minimum Inhibitory Concentration of tested essential oils after 3 days, 5 days, and 7 days against dermatophytes

Day	Essential oil (Name – Code)	MIC (% essential oil concentration)			p value
		Min	Max	Mean ± SD	
Day 3	*Cymbopogon citratus* - 1	0.02	0.08	0.05 ± 0.02	p (1,2), p (1,3), p (1,4), p (1,5): < 0.001
	Laurus cinnamomum - 2	0.04	0.15	0.1 ± 0.04	p (2,3) < 0.05 p (2,4), p (2,5): < 0.001
	Mentha arvensis L - 3	0.02	0.6	0.19 ± 0.15	p (3,4), p (3,5): < 0.01
	Melaleuca cajuputi - 4	0.15	1.25	0.43 ± 0.26	p (4,5) > 0.05
	Citrus grandis var. Thanh trà - 5	0.08	1.25	0.52 ± 0.34	
Day 5	*Cymbopogon citratus* - 1	0.02	0.08	0.05 ± 0.02	p (1,2), p (1,3), p (1,4), p (1,5): < 0.0001
	Laurus cinnamomum - 2	0.08	0.3	0.14 ± 0.07	p (2,3) < 0.01 p (2,4), p (2,5): < 0.001
	Mentha arvensis L - 3	0.02	0.6	0.28 ± 0.19	p (3,4), p (3,5): < 0.0001
	Melaleuca cajuputi - 4	0.3	1.25	0.66 ± 0.24	p (4,5) < 0.05
	Citrus grandis var. Thanh trà - 5	0.3	1.25	0.95 ± 0.36	
Day 7	*Cymbopogon citratus* - 1	0.04	0.15	0.08 ± 0.03	p (1,2), p (1,3), p (1,4), p (1,5): < 0. 001
	Laurus cinnamomum - 2	0.08	0.3	0.18 ± 0.08	p (2,3), p (2,4), p (2,5): < 0.01
	Mentha arvensis L- 3	0.02	1.25	0.5 ± 0.38	p (3,4), p (3,5): < 0.001
	Melaleuca cajuputi - 4	0.6	1.25	0.99 ± 0.33	p (4,5)> 0.05
	Citrus grandis var. Thanh trà - 5	0.6	1.25	1.09 ± 0.29	

(MIC: Minimum Inhibitory Concentration, SD: Standard Deviation)

At the 3-day time point, the average MIC of *Cymbopogon citratus* (lemongrass) was the lowest among the tested essential oils, with a statistically significant difference ($p < 0.0001$). The average MIC of *Laurus cinnamomum* (cinnamon) was lower than that of *Melaleuca cajuputi* (cajeput), *Mentha arvensis L* (peppermint), and *Citrus grandis var. Thanh trà* (pomelo)oil ($p < 0.05$). The average MIC of *Mentha arvensis L* (peppermint) was lower than that of *Melaleuca cajuputi* (cajeput) and *Citrus grandis var. Thanh trà* (pomelo) oil ($p < 0.001$). There was no statistically significant difference between the average MICs of *Melaleuca cajuputi* (cajeput) and *Citrus grandis var. Thanh trà* (pomelo)oil.

At the 5-day time point, the average MIC of *Cymbopogon citratus* (lemongrass) was the lowest among the tested essential oils, with a statistically significant difference ($p < 0.0001$). The average MIC of *Laurus cinnamomum (cinnamon)* was lower than that of *Melaleuca cajuputi* (cajeput), *Mentha arvensis L* (peppermint), and *Citrus grandis var. Thanh trà* (pomelo) oil ($p < 0.001$). The average

MIC of *Mentha arvensis L* (peppermint) was lower than that of *Melaleuca cajuputi* (cajeput) and *Citrus grandis var. Thanh trà* (pomelo) oil ($p < 0.001$). The average MIC of *Melaleuca cajuputi* (cajeput) was lower than that of *Citrus grandis var. Thanh trà* (pomelo) oil ($p < 0.05$).

At the 7-day time point, the average MIC of *Cymbopogon citratus* (lemongrass) was still the lowest among the tested essential oils, with a statistically significant difference ($p < 0.001$). The average MIC of *Laurus cinnamomum (cinnamon)* was lower than that of *Melaleuca cajuputi* (cajeput), *Mentha arvensis L* (peppermint), and *Citrus grandis var. Thanh trà* (pomelo)oil ($p < 0.01$). The average MIC of *Mentha arvensis L* (peppermint) was lower than that of *Melaleuca cajuputi* (cajeput) and *Citrus grandis var. Thanh trà* (pomelo)oil ($p < 0.001$). There was no statistically significant difference between the average MICs of *Melaleuca cajuputi* (cajeput) oil and pomelo. The detail data was showed in Table 1.

3.2. MIC of each essential oil against dermatophyte strain over the study periods

3.2.1. MIC value of *Cymbopogon citratus* (lemongrass) essential oil over the study period

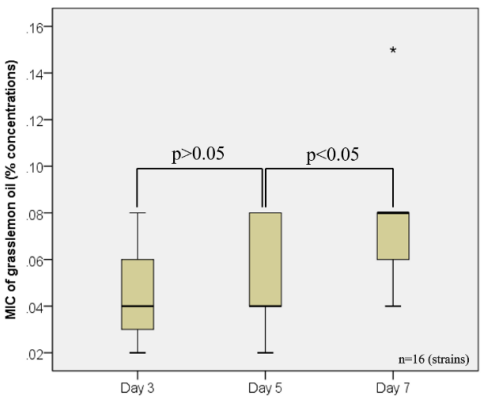

Figure 1. MIC value of *C. citratus* (lemongrass) essential oil to dermatophytes strains

The inhibitory ability of *Cymbopogon citratus* (lemongrass) essential oil remained stable with a median MIC value of 0.04% on days 3 and 5, and increased to 0.08% on day 7. No significant difference in MIC concentration was observed between days 3 and 5, but there was a significant difference between days 5 and 7 ($p < 0.05$). This indicated that the MIC concentration of the essential oil remained stable until day 5, but then increased afterward (**Figure 1**).

3.2.2. MIC value of *Laurus cinnamomum (cinnamon)* essential oil over the study period

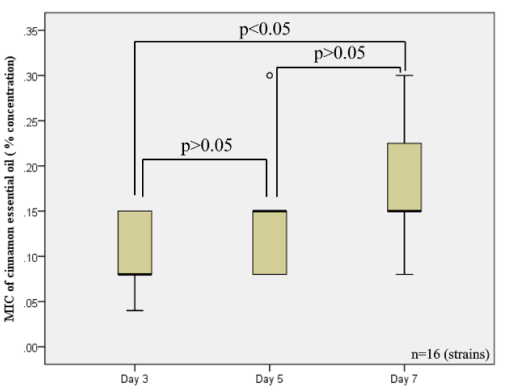

Figure 2. MIC value of *L. cinnamomum (cinnamon) oil* over to dermatophytes strains

The median MIC value of *Laurus cinnamomum (cinnamon)*essential oil was 0.08% on the 3rd day of the study and gradually increased over time, reaching a median MIC of 0.15% on days 5-7. There was a significant difference when comparing the MIC values between day 3 and day 7, indicating a substantial change in the MIC between the early and later stages of the study (**Figure 2**).

3.2.3. MIC value of Mentha arvensis L *(peppermint) essential oil over the study period*

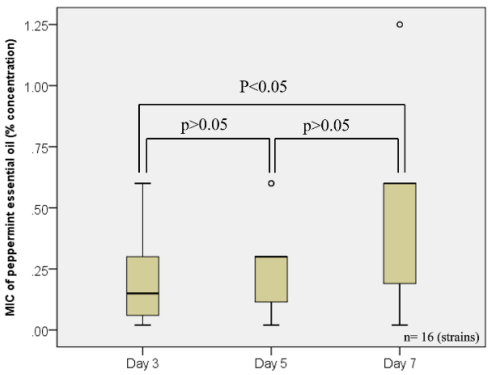

Figure 3. MIC value of *M. arvensis L* (peppermint) essential oil dermatophytes strains

The median MIC value of *Mentha arvensis L* (peppermint) essential oil increased over the survey periods and reached its highest level of 0.6% on the 7th day. Similar to *Laurus cinnamomum (cinnamon)* essential oil, no significant differences were observed between days 3-5 and 5-7 (p>0.05), while a significant difference was noted between days 3-7 (p<0.05) (**Figure 3**).

3.2.4. MIC value of Melaleuca cajuputi (cajeput) essential oil over the study period

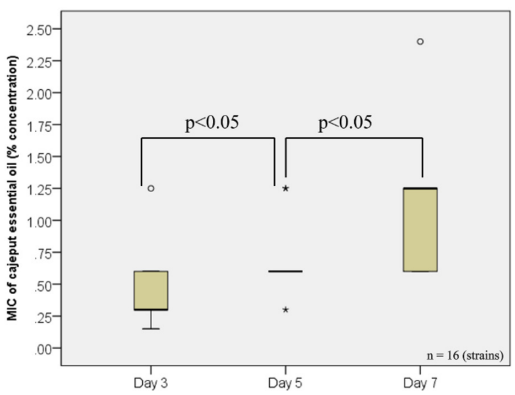

Figure 4. MIC value of *M. cajuputi* (cajeput) essential oil dermatophytes strains

The median MIC value of *Melaleuca cajuputi* (cajeput) essential oil increased over the study period, reaching its highest level of 1.25% on day 7. A significant difference was observed between days 3-5 and 5-7 (p<0.05), indicating that the concentration of this essential oil, despite having antifungal properties, was not stable over time (**Figure 4**).

3.2.5. MIC value of Citrus grandis var. Thanh trà (pomelo) essential oil over the study period

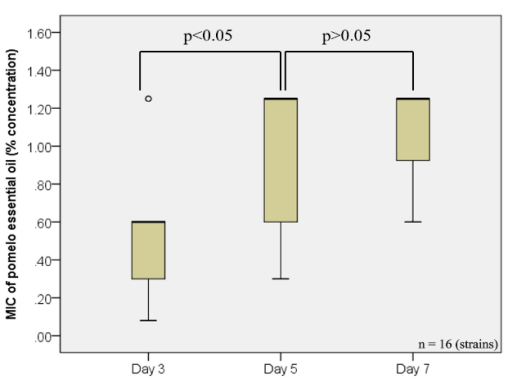

Figure 5. MIC value of *C. grandis var. Thanh trà* (pomelo)essential oil dermatophytes strains

The MIC median of *Citrus grandis var. Thanh trà* (pomelo)essential oil was higher than that of other essential oils at all surveyed time points, with median MIC values of 0.6%, 1.25%, and 1.25% at 3, 5, and 7 days, respectively. A significant difference in the MIC of *Citrus grandis var. Thanh trà* (pomelo)essential oil was observed between days 3 - 5 ($p < 0.05$), while no significant difference was found between days 5 - 7 ($p > 0.05$). This indicates that the inhibitory ability of the essential oil was unstable between days 3-5 but became stable from day 5 onward (**Figure 5**).

3.3. The MIC of the tested essential oils against T. indotineae after 3 days, 5 days, and 7 days

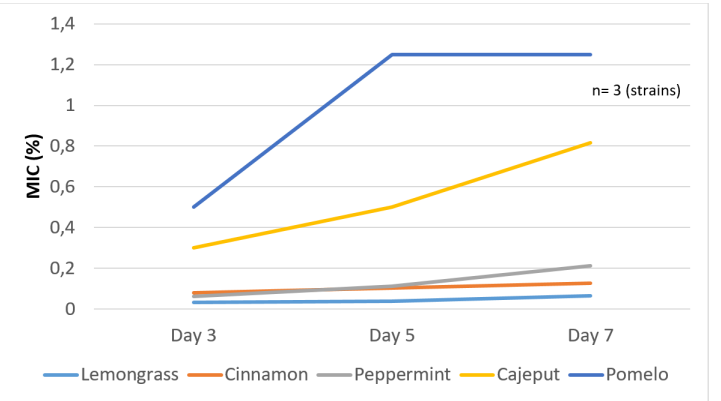

Figure 6. MIC of the tested essential oils against *T. indotineae* after 3, 5 and 7 days

Since the drug resistance of *T. indotineae* was more significant when compared to other species, and two third of the strains were resistant to terbinafine (these strains were reported under GenBanks accession numbers: OR366471 and OR366473 in the previous study [7]), we presented additional results on the minimum inhibitory concentration of the tested essential oils against *T. indotineae* after 3 days, 5 days, and 7 days. The results showed that *Mentha arvensis L* (peppermint), *Cymbopogon citratus* (lemongrass), and *Laurus cinnamomum (cinnamon)*essential oils effectively inhibited the *T. indotineae* strains, maintaining stable MIC concentrations throughout the 7-day incubation period, with values not exceeding 0.2%. Although *Citrus grandis var. Thanh trà* (pomelo)essential oil had a higher MIC, it maintained a stable MIC from day 5 onward (**Figure 6**).

4. DISCUSSION

During the 7-day incubation period, the results showed that all 5 essential oils were able to inhibit the growth of the tested dermatophyte species. This was consistent with findings from Nicole Parrish and co-authors' study, which also indicated that essential oils such as cinnamon, lemongrass, peppermint, and cajeput exhibited antifungal properties when cultured for up to 21 days [10], or with the report by Rodríguez VJ. on the beneficial effects of cinnamon essential oil on dermaphytosis [8].

Comparing the antifungal activity against dermatophytes among tested essential oils, our study found that *Cymbopogon citratus* (lemongrass), *Laurus cinnamomum (*cinnamon), and *Mentha arvensis L* (peppermint) essential oils had lower MIC values compared to *Melaleuca cajuputi* (cajeput) and *Citrus grandis var. Thanh trà* (pomelo) essential oils. Specifically, the MIC of lemongrass oil against dermatophytes was significantly lower than that of the other oils ($p < 0.001$). In Parrish's study, lemongrass oil was considered a moderate antifungal, and adding it every 3 days of incubation enhanced its antifungal efficacy compared to adding it only once as in our study. Another study by Capoci assessing the antifungal activity of lemongrass components against *M. canis* strains demonstrated that these substances have moderate to strong antifungal activity, showing their potential for preventing dermatophyte infections from animals to humans [15]. Our results also indicated that lemongrass oil exhibits antifungal activity against dermatophytes with a lower MIC value than cinnamon oil, and the stability of MIC values for lemongrass and cinnamon oils was almost the same during the study.

The effect of cinnamon essential oil on fungi has been reported in several studies. Parrish's research indicated that cinnamon oil is a potent antifungal agent when completely inhibits fungi even more effectively than antifungal drugs such as terbinafine and itraconazole in vitro. According to the systematic review by Rodríguez VJ., cinnamon

oil had a strong inhibitory effect on *Trichophyton rubrum* and *Trichophyton mentagrophytes* complex, reflected by low MIC values, indicating its potential for treating dermatophyte infections [8]. Experimental studies on guinea pigs by Ayatollahi Mousavi's team demonstrated that cinnamon effectively inhibits *M.canis, T.mentagrophytes*, *T.interdigitale,* and *M.gypseum*, applying this oil could also heal skin lesions within 9-11 days [16].

Additionally, our research shows that *Melaleuca cajuputi* (cajeput) oil, produced in several provinces in Vietnam including Hue City, and *Citrus grandis var. Thanh trà* (pomelo) oil, a specialty of Hue City, also have antifungal effects against dermatophytes. Notably, pomelo oil demonstrated superior MIC stability after 5 days of incubation compared to all other essential oils, marking a new finding in our study.

This study only examined the effects of essential oils on fungi and did not analyze the specific active compounds in each type of essential oil. Hence, this is a limitation of the study. Regarding the antifungal mechanism of essential oils, several studies have shown the diversity in how various natural essential oils kill and inhibit fungi, primarily depending on the types and ratios of compounds within each essential oil. Researchers have identified active compounds that play an important role in antifungal activity, such as terpineol, eugenol, linalool, citronellol, limonene, cinnamaldehyde, etc. These compounds affect fungi in multiple ways, including damaging the cell wall and membrane, causing mitochondrial injury, and inhibiting efflux pumps, among others [17].

According to the information provided by the manufacturer, we noted that some of the active compounds in our essential oils have been researched for their antifungal mechanisms. For example, geraniol in *Cymbopogon citratus* (lemongrass) oil has been shown to inhibit the transcription of the *CaCdr1p* gene, thereby inhibiting the activity of efflux pumps. Efflux pumps are transport proteins that pump drugs and harmful substances out of the cytoplasm of fungi. Inhibiting these pumps disrupts the intracellular environment and therefore kills the fungi. Similarly, cinnamaldehyde in *Laurus cinnamomum (cinnamon)* oil can not only inhibit efflux pumps but also damage the fungal cell wall because of its hydrophobicity that allows it to easily pass through the fungal cell wall and membrane, altering the fluidity and permeability of the plasma membrane. That results in cytoplasmic coagulation and fungal death. Compounds in the terpene family, such as myrcene (found in lemongrass and pomelo oil), alpha-pinene (cajeput and pomelo), terpinolene (cajeput), and terpinene (pomelo), can penetrate the cell membrane and accumulate in the fatty acid chains of the lipid bilayer, thereby damaging the structure of the fungal plasma membrane. Another mechanism noted is that of limonene, found in pomelo oil, inhibiting enzymes in the tricarboxylic acid (TCA) cycle, causing mitochondrial dysfunction in fungi [18].

The analysis of the essential oils'MIC value stability over different culture periods showed that lemongrass, peppermint, and cinnamon essential oils had stable MIC concentrations between day 3 and day 5, but then increased by day 7. In contrast, pomelo essential oil, despite having a high MIC value, remained stable after 5 days. In the study of Parrish et al, many essential oils demonstrated strong activity during the first 10 days; however, after 21 days, the fungi could regrow. Therefore, another limitation of our study was that it only evaluated up to 7 days, and further research with longer study periods is necessary.

The resistance of *T. indotineae* to terbinafine, the first-line drug in the treatment of dermatophyte infections, was first reported in India and later in many countries around the world including Vietnam [5], [6], [7]. Therefore, studying the effects of essential oils on *T. indotineae* is both novel and practical. Our results showed that peppermint, lemongrass, and cinnamon essential oils all effectively inhibited these fungal strains, maintaining MIC concentrations within 7 days of cultivation that did not exceed 0.2%. This finding is particularly significant, as 2 out of the 3 strains surveyed were resistant to terbinafine [7].

Based on the current research results and the studies discussed, more research is needed to investigate the antifungal activity of essential oils originating from various regions in Vietnam as well as from different countries on dermatophytes with a broader range of strains and fungi types. Longer testing periods are also necessary to more accurately assess the antifungal effects of these essential oils. Additionally, it is important to explore whether combining different essential oils could enhance their antifungal potency, or whether incorporating essential oils into topical antifungal preparations could improve therapeutic efficacy.

5. CONCLUSION

This is the first in vitro study in Vietnam that evaluated the antifungal activitiy of essential oils to dermatophyte strains. The primary results indicated that all tested essential oils exhibited inhibitory

activity against dermatophytes. These results also showed that *Cymbopogon citratus* (lemongrass) oil exhibited superior antifungal activity compared to the other tested oils and maintained efficacy over a longer period. *Laurus cinnamomum (cinnamon)* and *Mentha arvensis L* (peppermint) oils also showed significant antifungal activity, while *Citrus grandis var. Thanh trà* (pomelo) oil, although having higher MIC values, maintained stability in antifungal efficacy over the assessed period. Notably, *Mentha arvensis L* (peppermint), *Cymbopogon citratus* (lemongrass), and *Laurus cinnamomum (cinnamon)* oils effectively inhibited fungal strains of *T. indotineae*, and kept MIC values below 0.2%.

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