Clinical, Cosmetic and Investigational Dermatology

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ORIGINAL RESEARCH

Comparison of in vitro Killing Effect of Thai Herbal Essential Oils, Tea Tree Oil, and Metronidazole 0.75% versus Ivermectin 1% on *Demodex folliculorum*

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Background: Abnormal proliferation of *Demodex* mites causes a skin disorder called demodicosis and has been linked to rosacea. The development of alternative therapy against *Demodex* mites is currently required. The ability to kill *Demodex* mites of Thai herbal essential oils has never been explored. This study aimed to study and compare the in vitro killing effect of Thai herbal essential oils, tea tree oil, and metronidazole 0.75% with ivermectin 1% on *D. folliculorum*.

Materials and Methods: *D. folliculorum* mites were collected from the wastes of diagnostic standardized skin surface biopsy samples of demodicosis and rosacea patients for the trial. The microscopic evaluation started immediately after the mites were exposed to immersion oil (negative control), Thai herbal essential oils, tea tree oil, metronidazole 0.75%, and ivermectin 1% (positive control). The survival times of ten mites from each test agent were compared.

Results: The efficacy of Thai herbal essential oils and other test agents can be arranged in order as follows: lemongrass oil > sweet basil oil > clove oil > tea tree oil > lesser galangal oil > ginger oil, kaffir lime oil, peppermint oil > citronella oil > galangal oil > cajeput oil > ivermectin 1% > metronidazole 0.75%.

Conclusion: This current study demonstrated the in vitro killing efficacy on *D. folliculorum*: Thai herbal essential oils, Tea tree oil > ivermectin 1% > metronidazole 0.75%. Thai herbal essential oils have the potential to be an adjuvant or alternative therapy against *Demodex* mites. Further in vivo studies are necessary to determine the treatment efficacy and side effects.

Keywords: essential oils, metronidazole, ivermectin, *Demodex* mites, demodicosis, rosacea

Introduction

Demodex mites are tiny parasites living in the pilosebaceous glands of humans and animals. They are classified as arachnids, the same as spiders and ticks. Even though there are more than one hundred species, only two can be found in humans. *D. folliculorum* usually lives in the hair follicle, and *D. brevis*, which has a shorter opisthosoma, typically resides in the sebaceous gland and duct. Both species are commonly found on the face, cheeks, perinasal area, eyelashes, and eyebrows.[1,2]

Abnormal proliferation of *Demodex* mites causes a skin disease called demodicosis. In addition, several studies demonstrated a significant association between *Demodex* mites and rosacea.[1–4]

Many medicines and acaricidal agents have been reported to be effective in reducing the abnormal proliferation of *Demodex* mites and improving clinical symptoms of patients with demodicosis and rosacea. The oral treatments are metronidazole and ivermectin, while the common topical treatments include metronidazole, permethrin, ivermectin, crotamiton, lindane, and benzyl benzoate.[4,5] Despite many treatment modalities, resistance and relapse to the treatment of demodicosis are reported.[6,7] Moreover,

Received: 13 April 2023
Accepted: 16 May 2023
Published: 18 May 2023

the side effects of topical and oral medication are also common.[5] For all these reasons, developing new alternative approaches against *Demodex mites* is necessary.

Thai people have used herbal essential oils for hundreds of years. We use it as an ingredient in food, perfume, cosmetics, insect repellent, and topical anti-ectoparasite. Many studies revealed the acaricidal effect on *Sarcoptes scabies, Pediculus humanus capitis*, and Ticks of essential oils.[8–10] Tea tree, sage, thyme, and salvia oil have been tested for the killing effect on *Demodex* mites.[11–14] The ability to kill *Demodex* mites of Thai herbal essential oils has never been explored, as well as metronidazole 0.75% and ivermectin 1%, commonly used in rosacea and demodicosis.

This study aimed to study and compare the in vitro killing effect of Thai herbal essential oils, tea tree oil, and metronidazole 0.75% with ivermectin 1% on *D. folliculorum.*

Materials and Methods

Demodex Mites

We included 140 adult *D. folliculorum* mites from the wastes diagnostic standardized skin surface biopsy (SSSB) slides from 140 patients who presented to our clinic and were diagnosed with demodicosis and rosacea. We selected the most active mite from each slide as the subject of this study. The waste diagnostic SSSB slides from patients who had received topical or systemic treatment with antibacterial or acaricidal agents in the last three months were excluded.

The study protocol was approved by Mae Fah Luang University Ethics Committee with approval number COE 007/2023.

Materials

All essential oils in this study were obtained by distillation, except kaffir lime oil was acquired by expression. One hundred percent pure essential oil of clove (*Syzygium Aromaticum*), cajeput (*Melaleuca Cateputi*), peppermint (*Mentha Piperita*), galangal (*Alpinia Galanga (Linn.) Swartz*), lesser galangal (*Boesenbergia Pandurata (Roxb.) Schltr.*), citronella (*Cymbopogon Nardus*), lemongrass (*Cymbopogon Flexuosus*), ginger (*Zingiber Officinale Rose*), sweet basil (*Ocimum Basilicum*), kaffir lime (*Citrus Hystrix*), and tea tree oil (*Melaleuca Alternifolia*), are products from Chemipan co., LTD. (Thailand). Metronidazole gel 0.75% is manufactured by Laboratoires Galderma (France). Ivermectin 1% solution is produced by Bukalo trading co., LTD. (Thailand). Immersion oil is a Hollywood International co., LTD. (Thailand) product.

Methods

The experimental design was completely randomized with ten *Demodex* mites for each test agent (only one mite from each SSSB slide). The positive control group was exposed to ivermectin solution 1%, while the negative control group was exposed to immersion oil.

The test groups were directly exposed to metronidazole gel 0.75%, the essential oils of clove, cajeput, peppermint, galangal, lesser galangal, citronella, lemongrass, ginger, sweet basil, kaffir lime, and tea tree oil. The microscopic evaluation was initiated immediately after the mites were contacted with test agents. The viability of *Demodex* mites was evaluated for a total of 240 min by periodic observation of each slide through the microscope. The motility of the mites was observed at different time intervals. The observation period was 1 min in every 2 min of the first hour and after that, every 10 min during the second hour, every 20 min during the third hour, and every 30 min during the fourth hour of the study. Mites were considered dead when no head, body, and leg movement was seen over 2 min even after being provoked by pressing on the slides with a needle. The survival time (ST) is the interval between the first mite exposure with the test agents and the time total body movements are discontinued.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). Numerical variables were shown as a median, interquartile range, mean, and standard deviation (SD). The survival analysis using the Kaplan–Meier method demonstrated ST between the test agents. A Log rank test evaluated the comparison of ST between groups; a p-value less than 0.05 was considered significant in all comparisons.

Results

All *D. folliculorum* mites died within 16 min after exposure to Thai herbal essential oils and tea tree oil compared to 20 min after exposure to ivermectin 1%. After exposure to lemongrass, sweet basil, clove, and tea tree oil, all mites died within 2, 4, 4, and 6 min, respectively.

In the metronidazole 0.75%, six mites died within 120, 140, 160, 180, 180, and 210 min, respectively, while the remaining four mites survived until the end of observation. All mites survived in immersion oil for the entire observation period of 240 min.

Based on their mean ST in different test agents (Table 1), the mite-killing efficacy can be arranged in the following order: lemongrass oil > sweet basil oil > clove oil > tea tree oil > lesser galangal oil > ginger oil, kaffir lime oil, peppermint oil > citronella oil > galangal oil > cajeput oil > ivermectin 1% > metronidazole 0.75%.

The differences between the median ST in all Thai herbal essential oils and tea tree oil were statistically significantly shorter than immersion oil and ivermectin 1% ($p < 0.001$). While the median ST in metronidazole 0.75% showed significantly shorter than immersion oil ($p = 0.0041$) but longer than ivermectin 1% ($p < 0.001$) (Table 2).

Table 1 The Survival Time *of D. folliculorum* from Standardized Skin Surface Biopsy Slides in Different Test Agents

Test Agents	n	Median	IQR	Mean	SD	Min	Max
Lemon grass essential oil	10	2	–	2.0	0.0	2	2
Sweet basil essential oil	10	2	–	2.2	0.6	2	4
Clove essential oil	10	2	–	2.4	0.8	2	4
Tea tree essential oil	10	2	–	2.6	1.3	2	6
Lesser galangal essential oil	10	8	6–10	8.2	2.0	6	12
Peppermint essential oil	10	8	6–10	8.6	2.3	6	12
Ginger essential oil	10	8	8–10	8.6	1.9	6	12
Kaffir lime essential oil	10	8	8–10	8.6	2.1	4	12
Citronella essential oil	10	8	8–10	8.8	1.7	6	12
Galanga essential oil	10	10	8–12	10.4	2.8	8	16
Cajeput essential oil	10	10	10–12	10.6	2.3	8	16
Positive control (ivermectin 1%)	10	16	16–18	17.2	1.9	14	20
Metronidazole 0.75%	10	180	–	195.0	45.5	120	240
Negative control (immersion oil)	10	–	–	240.0	0.0	240	240

Abbreviations: IQR, interquartile range; SD, standard deviation.

Table 2 To Compare the Median Survival Time Differences Between Thai Herbal Essential Oils and Tea Tree Oil, Metronidazole 0.75%, Positive Control (ivermectin 1%), and Negative Control (Immersion Oil), Using the Log Rank Test

Test Agents	P value*
Positive control (ivermectin 1%) versus negative control (immersion oil)	<0.001
Thai herbal essential oils and tea tree oil versus negative control (immersion oil)	<0.001
Thai herbal essential oils and tea tree oil versus positive control (ivermectin 1%)	<0.001
Metronidazole 0.75% versus negative control (immersion oil)	0.0041
Positive control (ivermectin 1%) versus metronidazole 0.75%	<0.001

Note: *Log Rank Test was used to compare the survival time between the test agents.

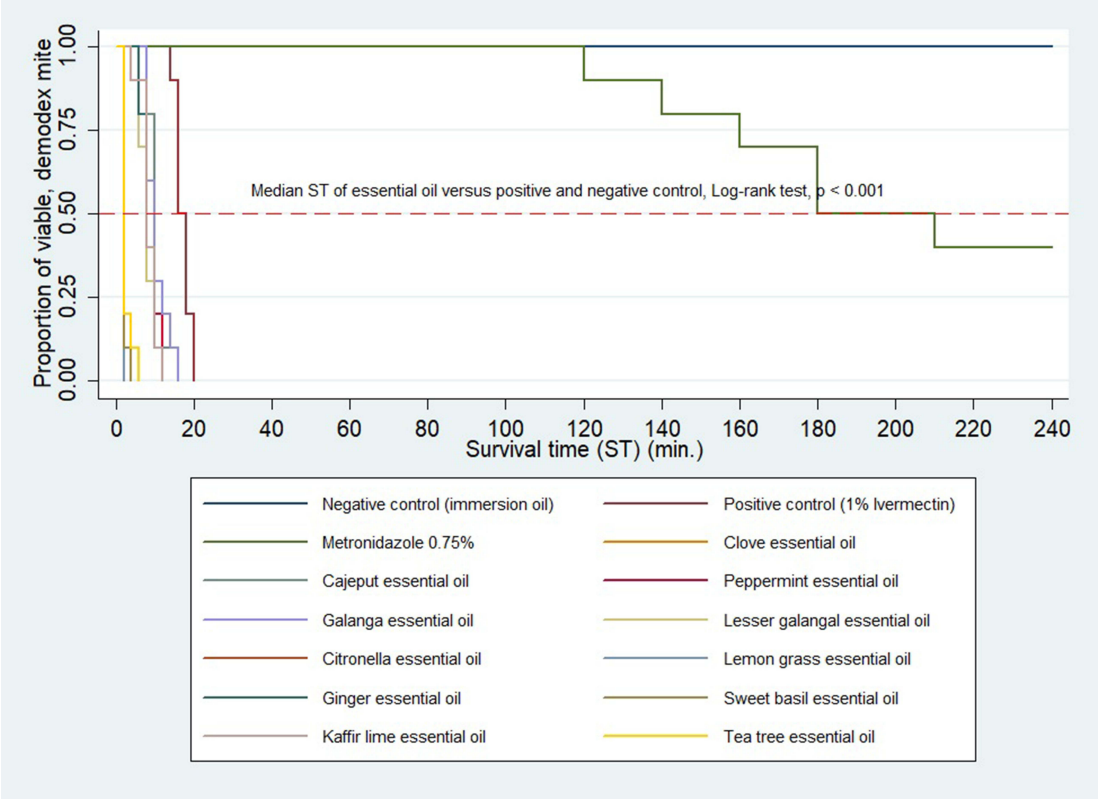

Figure 1 Kaplan-Meier survival curve to demonstrate the survival time between the test agents.
Abbreviation: ST, survival time.

Kaplan–Meier cumulative *D. folliculorum* survival curves of different test agents are presented in Figure 1. A comparison of the differences in the median ST between test agents using the Log rank test is shown in Figure 2.

Discussion

Essential oil is a concentrated pure oil containing volatile chemical compounds from plants that evaporate quickly at room temperature. Available literature reported that essential oil is mainly used to treat skin infections caused by fungi, viruses, and bacteria, followed by inflammatory skin diseases, such as eczema, acne, burn, wound, and psoriasis; the third most common are cosmetic purposes such as wrinkles and scars.[15]

Although the efficacies of essential oil against *Demodex* mites have already been reported, Thai herbal essential oils have yet to be discovered.[11,13,14] For the first time, the current study allowed us to study and compare Thai herbal essential oils, tea tree oil, and metronidazole 0.75%, with ivermectin 1%.

All mites died within 16 min after exposure to Thai herbal essential oils, indicating the strong in vitro killing effect of these oils, especially lemongrass, sweet basil, and clove oil, that killed mites within 4 min of exposure.

From the literature review, we found that lemongrass oil consists of β-geranial, Z-citral, and geraniol, which have the effect of anti-inflammatory, antifungal, and anti-parasite by destroying the cell membrane integrity and permeability of parasites.[16,17] Recently, lemongrass oil was discovered to be a promising miticidal and ovicidal agent against *Sarcoptes scabiei*.[18] Sweet basil oil has linalool and estragole (methyl chavicol) and can be a potential antioxidant, antimicrobial, acaricidal, and anthelmintic agent against ticks and helminth parasites.[19–22] Clove oil contains a main composition of phenylpropanoids such as carvacrol, thymol, eugenol, and cinnamaldehyde with antimicrobial, insect-repellent, anesthetic properties and acaricidal activity against scabies mites.[23,24] To the authors' knowledge, this is the first study to assess the in vitro killing effect of lemongrass, sweet basil, and clove oil on *Demodex* mites.

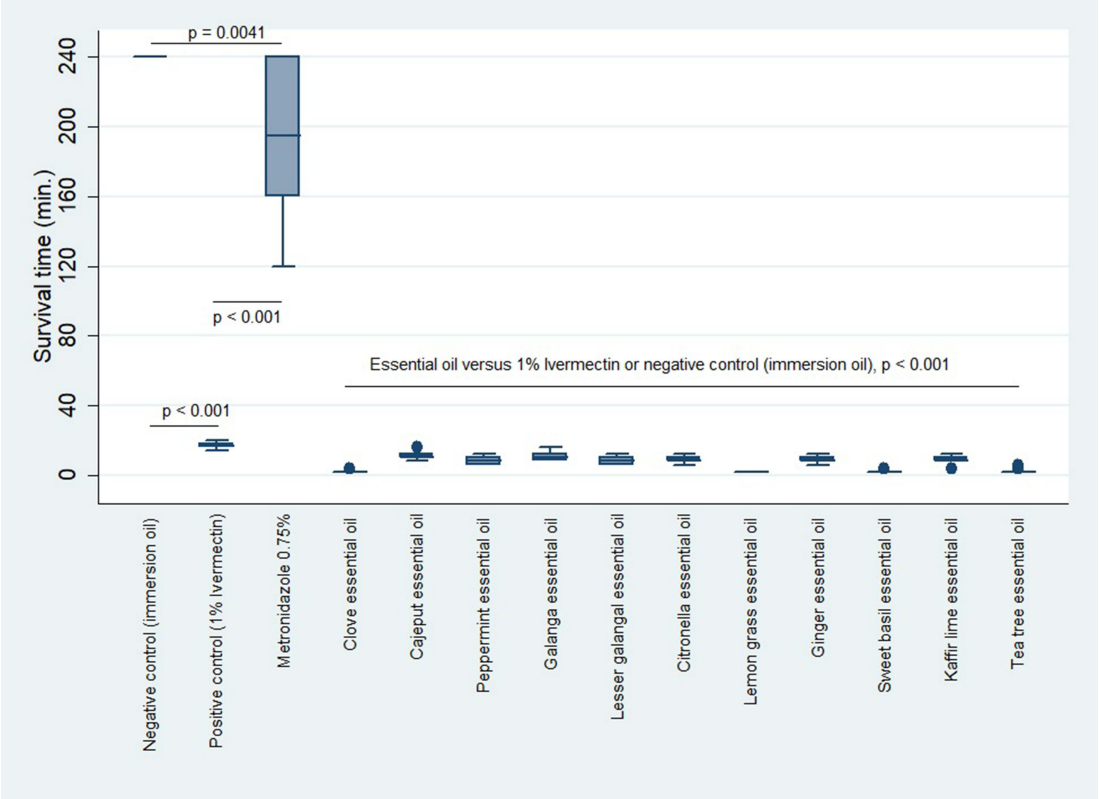

Figure 2 To compare the median survival time difference between the pairs using the Log rank test.

In contrast to tea tree oil, several studies have demonstrated that a possible mechanism of action of tree tea oil against *Demodex* mite is the inhibition of acetylcholinesterase by terpinen-4-ol (T4O).[5,11,25] Compared to previous studies, the median ST of *Demodex* mites in tea tree and peppermint oil was reported at 3.3 min and 11 min. In contrast, in this study, they were 2 min and 8 min, respectively[11,14] The heterogeneity in the study results could be explained by differences in the chemical composition of the essential oils, which may be due to the different chosen plant parts, species, origin, cultivation date, and extraction methods.[26]

Metronidazole is a unique drug initially developed for treating parasites such as *Trichomonas vaginalis, Entamoeba histolytica*, and *Giardia lamblia* but later used extensively in treating bacterial infections. Metronidazole causes cell death by diffusing into the organism, inhibiting protein synthesis by interacting with DNA, and causing loss of DNA helix structure.[27]

The efficacy of topical metronidazole in rosacea is described by an anti-inflammatory action and decreases the follicular density of *Demodex* mites without directly killing them.[28] In the present study, the ability to kill *Demodex* mites of metronidazole 0.75% was demonstrated for the first time. Although the efficacy is not as good as ivermectin and essential oils, the mean ST of *Demodex* mites in metronidazole 0.75% was significantly shorter than immersion oil (negative control).

Topical ivermectin has been reported for its efficacy in patients with ocular *Demodex* and rosacea associated with *Demodex* mites.[29,30] Ivermectin binding with g-aminobutyric acid–gated chloride ion channels in the peripheral synapses of neurons leads to paralysis and death of *Demodex* mite.[31] Although several human studies have been conducted, this is the first in vitro study showing good efficacy against *Demodex* mite of topical ivermectin 1%.

Despite not being included in the objectives of this study, we noticed two interesting findings from the experiment. First, mites exposed to essential oil shrank, distorted, and deformed within a few minutes after death, while mites exposed to ivermectin became smaller and translucent two hours later (Figure 3). These findings demonstrate differences in the mechanism of action between essential oils and ivermectin. Second, essential oils can seep into follicular keratin

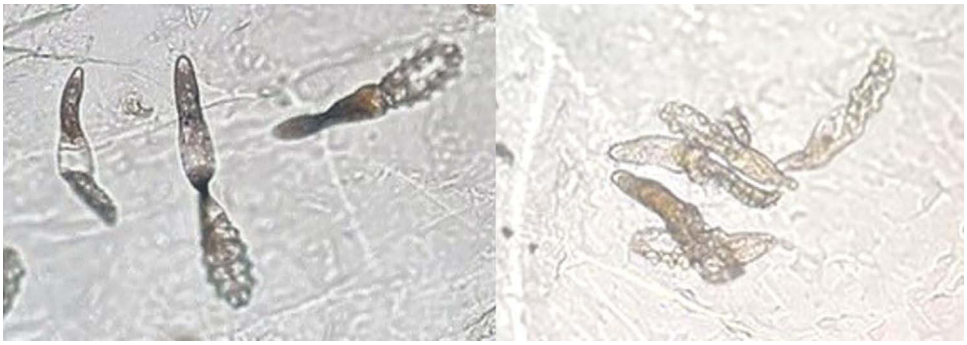

Figure 3 Mites exposed to sweet basil oil shrank, distorted, and deformed after a few minutes of death, while those exposed to ivermectin became smaller and translucent two hours later.

Figure 4 Two mites covered with follicular keratin stopped moving and died within 2 min after exposure to lemongrass oil. The mites shrank, distorted, and became transparent within 30 min after death.

and kill the mites that hide inside (Figure 4). Follicular keratin is a perfect shield for mites, making them difficult to be killed by acaricidal agents. This unique ability could make essential oils an ideal agent against *Demodex* mites.

Our findings and previous studies showed strong evidence that essential oil had a potentially effective acaricidal agent for *Demodex* mite.[11,13,14] The mechanism of action of essential oils against mites differs from ivermectin and metronidazole. Thai herbal essential oils, especially lemongrass, sweet basil, clove oil, and tea tree oil, should be considered an alternative acaricidal agent against *Demodex* mites.

The long history of using essential oils gives us a sense of safety as a natural product. Nevertheless, there have been reports of allergies and skin eruption from essential oil.[32,33] Therefore, to prevent side effects that may occur, lowering the concentration and eliminating the main allergens from the original essential oils are important.

Further studies should consider identifying active compounds of each Thai herbal essential oil against *Demodex* mites and their mechanism of action, the appropriate concentration, and formulation. In addition, in vivo studies are necessary to determine the efficacy of killing *Demodex* mites, and potential side effects should also be a major concern.

Limitations of the Study

The limitations of this study are the small number of *Demodex* mites in each test group. Differences in age, sex, and size of *Demodex* mites may affect the mite-killing effect of test agents. The long interval between each observation through the microscope during the third hour and the fourth hour of the study may effect on ST of *Demodex* mite in metronidazole 0.75%.

Conclusion

This study demonstrated that Thai herbal essential oils and tea tree oil had a superior in vitro killing effect than ivermectin 1%, while ivermectin 1% was better than metronidazole 0.75%. Thai herbal essential oils are potentially an adjuvant or alternative therapy against *Demodex* mites. Further in vivo studies are necessary to determine the treatment efficacy and side effects.

Data Sharing Statement

Unavailable data, but the reader can personally request access via Dr Anon Paichitrojjana; E-mail: anonpaic@gmail.com.

Statement of Ethics

The study was conducted in accordance with the World Medical Association Declaration of Helsinki. The study protocol was reviewed and accepted by The Mae Fah Luang Ethics Committee on Human Research approval, with reference number COE 007/2023.

Acknowlegment

All authors thank the School of Anti-aging and Regenerative Medicine, Mae Fah Luang University, for its research facilities.

Funding

This study did not receive any funding.

Disclosure

The authors report no conflicts of interest in this work.

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