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The Efficacy of Calendula Officinalis Extract on the Mortality of Demodex Folliculorum

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ABSTRACT

Background

Calendula officinalis is believed to exhibit antimicrobial and anti-inflammatory properties. Consequently, it was evaluated for Demodex folliculorum mite which is associated with several dermatosis. Many available treatments have some demerits and there is growing demand for alternative therapies. Till now there is no in-vitro experiment of Calendula on mite. This investigational study aimed to highlights the effect of Calendula on Demodex.

Objective

To study the efficacy of Calendula officinalis flower extract on the mortality of Demodex folliculorum in-vitro.

Method

Demodex mites were collected from 44 healthy participants (alive mites reported in 40 participants) having oily complexion and demodicosis-affected skin using skin scraping, squeezing, and SSSB. Various concentration of Calendula extract, 1% ivermectin, coconut oil and immersion oil were then applied to Demodex. Each chemical was randomly introduced to ten mites. Under microscopic observation, complete immobility following needle stimulation indicated mortality. Groups were compared on survival time (ST) from chemical exposure to full immobility. The experiment was repeated three times to ensure accuracy and a reliable comparison of chemical's effect on mite survival.

Result

In the in-vitro investigation, the flower extract of Calendula officinalis had no direct parasiticidal effect on the Demodex even at concentration 100% w/v. Thus, it was inferior to ivermectin in mite eradication. Coconut oil also demonstrated similar effect. The efficacy of acaricidal agents against Demodex is ranked as follows: ivermectin 1% > Calendula officinalis = coconut oil = immersion oil.

Conclusion

This experimental study demonstrated that Calendula officinalis flower extract lacks significant acaricidal effects on Demodex folliculorum. Hence, Calendula extract cannot be utilized as a direct anti-Demodex agent in demodicosis. Nevertheless, anti-inflammatory properties of Calendula from previous studies might control inflammation a primary factor disrupting skin homeostasis. Hence, Calendula may serve as a supportive therapy in maintaining parasitostasis on skin, without eliminating Demodex.

KEY WORDS

Acaricides, Calendula, Demodex, Demodicosis, Mortality, Survival time

INTRODUCTION

Calendula officinalis, a common aromatic herbal flowering plant popularly known as English marigold or pot marigold is often utilized in several medical and cosmetics purposes. The ubiquitous annual plant belongs to the Asteraceae family and has been indigenous to the Mediterranean, Macaronesia, Western Europe, and Southwest Asia but has adapted well to many regions around the world.[1,2]

The flowering plant has been extensively utilized in the field of medicine and cosmetics to treat a wide variety of diseases. Calendula is known for its diverse pharmacological therapeutics in the fields of dermatology.[3,4] Topically, people apply it to heal wounds, reduce inflammation, and soothe irritated tissue. It helps to manage acne by reducing inflammation and controlling bleeding.[5] People also use the plant to treat eczema, as it soothes the skin and reduces irritation.[6] Through tissue repair, Calendula oil, known for its wound-healing capabilities, treats skin injuries such as skin damage, scars, frostbite, burns, and ulcers.[7] When applied as an infusion, it is beneficial for eye inflammation and bee stings.[8] Clinical tests have shown that cosmetic formulations containing Calendula extract cause minimal irritation or sensitization, making them suitable for sensitive skin.[9] The plant's rich content of active biological ingredients such as flavonoids, saponins, methanol, triterpenes and carotenoids contribute to its effectiveness in treating various skin diseases.[10,11] Most importantly, the plant has been assumed to have antimicrobial activities because of its potent phytochemicals.[12-14]

Besides, the herbal plant also exhibits a wide range of biological activities, such as anti-inflammatory, analgesic, anti-diabetic, and ulcer-preventive properties, and it also serves as a treatment for burns, gastrointestinal disorders, gynecological issues, and ocular diseases.[10,15,16] Demodex folliculorum residing in the pilosebaceous unit of skin feeds on sebum, cellular debris and keratin thus lives in harmony with other skin flora.[17] Under certain circumstances like change in ph. of skin, microbiota density, gut-skin axis, bodily immunity, skin integrity restoration status, the proliferation of the Demodex mite seems to cause wide diverse clinical dermatosis which are collectively called as demodicosis.[18] Papulo-pustular rosacea and acneiform eruption are some of the common associated diseases mentioned in the literature.[19,20] Despite their availability, treatments yield suboptimal results owing to undesirable side effects and elevated costs. Besides, some cases of ivermectin resistance have also been found.[21] As result there is growing need of alternative treatment for Demodex mite control and Calendula can be one option for this since previous studies have reported the anti-inflammatory benefit on human skin in controlling inflammation, a key factor that promotes mites proliferation.

The efficacy of Calendula in the treatment of Demodex mites remains poorly understood. Most of the available data is informal and focusses on broad antimicrobial properties rather than specific acaricidal behaviors. Clinical research directly assessing its efficacy in eradicating Demodex mites are few. There is limited evidence regarding the effects of Calendula on mites, as well as its effective formulations and concentrations. No studies have been undertaken to evaluate its effectiveness in treating demodicosis. Furthermore, there are no clinical trials on human Demodex comparing its efficacy with other well recognized treatments like ivermectin, metronidazole, tea tree oil etc. The phytochemicals in Calendula that could potentially possess acaricidal capabilities are yet to be determined. Further investigation is required for clarification of its mechanisms and possible advantages. Methodically designed research investigations are crucial to validate its efficacy. This study was initiated to evaluate the efficacy of Calendula's floral extract against Demodex and determine the appropriate concentration for therapeutic usage in facial dermatoses.

METHODS

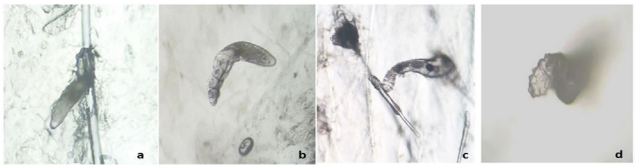
All the chemical (Calendula extract, ivermectin, coconut oil, immersion oil etc.) required for the research study was obtained from different registered manufacturing companies with valid manufacturing date. Calendula officinalis tincture (100 ml) and gel with Batch No. UM210994 was obtained from SBL Pvt Ltd, Homeopathy company, India. Similarly,1% ivermectin 1% w/v 100 ml having batch no. IUM0440823 was used from the TCI Laboratories CO. Ltd, USA. Additionally, 500 ml coconut oil was produced from the company Judcha Ee Coco Care Co, Ltd, Thailand and 40 ml immersion oil was obtained from LOBA Chemie company, Thailand. The microscope mobilized for the visualization of Demodex mite was from Microscope company (Nikon Eclipse E100, Japan).

The research design involved complete randomization of Demodex mites, comprising a group of ten mites for each chemical being tested. The chemicals tested included Calendula officinalis, ivermectin, immersion oil and coconut oil. Altogether, a total of 352 slides from 44 patients (alive mites reported among 40 participants) were considered and the mites were extracted using various procedures such as squeezing, skin scraping, and Standardized Skin Surface Biopsy (SSSB) (Fig. 1). Only the viable, active, and motile adult Demodex folliculorum mites were chosen for the investigation, 1% ivermectin solution (positive control) and immersion oil (negative control) were used to compare how well Calendula officinalis worked.

The actively motile Demodex folliculorum mites (Fig. 3: a-d) were directly exposed to Calendula officinalis and the duration of exposure was documented. The microscopic inspection was commenced immediately following exposure to testing chemicals. Through careful examination of each glass slide under a microscope (Fig. 2), the Demodex mites' survival time was tracked for a total of 360

Graph 1. **Demodex extraction by SSSB**

Graph 2. **Mites observation under microscope**



Graph 3 a-d. **Demodex folliculorum :10X magnification under microscope**

minutes. The mobility of the mites was monitored carefully at various time intervals. The observation interval was every 2 minutes during the first hour, followed by every 10 minutes during the second hour, every 20 minutes during the third hour, and every 30 minutes during the fourth, fifth and sixth hour of the study. Mites were considered dead when there was an absence of movement of the body or legs after a thorough observation under microscope for 1 minute despite manipulation with a fine needle. Survival time (ST) denotes the interval between the initial chemical exposure and the complete cessation of all observable mite movement.

Statistical analysis was performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed in the form of interquartile range (IQR), mean, and standard deviation (SD) and inferential statistics were represented by Kaplan–Meier survival curve. Log rank test was utilized for the comparison of ST between groups; a p-value less than 0.05 was considered significant in all comparisons.

RESULTS

The in-vitro experimental study discovered that the all the mites treated with the floral extract of the Calendula officinalis remained alive despite application of chemicals multiple times. This happened to even maximum concentration (100% w/v) of Calendula officinalis. The results showed that there are no lethal effects of Calendula plant on the Demodex folliculorum mites. This might be due either to the lack of a higher dose or to the absence of a chemical capable of penetrating the chitinous layer and producing a cytotoxic effect. In contrast l% ivermectin which was utilized as positive control produced mortality effect on Demodex mite within an average time of 26 minutes. The range of ST in different Demodex mite in 1% ivermectin group ranged from 18-42 minutes. Similar effect like Calendula was observed on two other chemicals: coconut oil and the immersion oil which was utilized as negative control. Coconut oil which was thought to possess antimicrobial potential has no lethal effect on the Demodex folliculorum throughout the experimental time of 360 minutes. All the mites exposed to immersion oil and coconut oil showed active crawling movement even after the period of 360 minutes. Mites that remained alive even after the designated experimental duration were discarded and classified as part of the censored group. Based on the survival time (ST) (Table 1) the Demodex killing potential of these testing chemical can be arranged in following order: ivermectin 1% > Calendula officinalis = coconut oil=immersion oil. The survival time (ST) of mites exposed to Calendula officinalis was not substantially reduced when compared to those mites subjected to 1% ivermectin, as evidenced by a p-value exceeding 0.05. Similarly, there is no statistical difference in the ST of the mites when compared among calendula, immersion oil and coconut oil (Table 2).

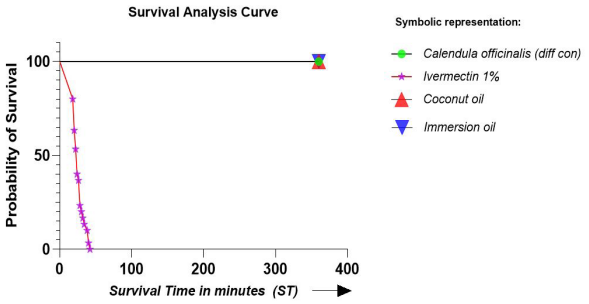
Table 1. **Descriptive studies of the mean survival time.**

Test chemicals	Sample n	Mean Survival time (ST) in minutes	Standard Deviation (SD)	Minimum	Maximum	Censored (Dead/ Alive)
Calendula officinalis	30	360.00	.000	360	360	Alive (discarded)
Ivermectin 1%	30	25.47	7.295	18	42	Dead
Immersion oil	30	360.00	.000	360	360	Alive (discarded)
Coconut oil	30	360.00	.000	360	360	Alive (discarded)
Total	120	304.24	125.055	18	360	

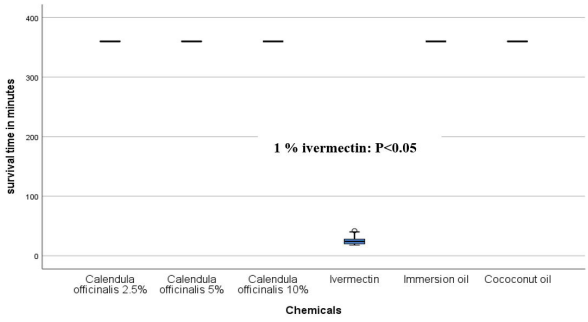
Table 2. **Statistical significance comparison among test chemicals:**

Test Agents: Chemicals	p-value
Ivermectin 1% (Positive control) versus (immersion oil) negative control	<0.05
Calendula officinalis (Diff conc) versus (ivermectin 1%) positive control	<0.05
Calendula officinalis (Diff conc) versus (immersion oil) negative control	1.000
Calendula officinalis (Diff conc) versus coconut oil	1.000
(Ivermectin 1%) positive control versus Coconut oil	<0.05
Coconut oil (Diff conc) versus (immersion oil) negative control	1.000

The graphical representation of ST of Demodex mites against 4 different testing chemicals has been illustrated on the Kaplan-Meier Curve in figure 4. Similarly, the comparison of the survival time of the Demodex mites at the end of the experimental period in the laboratory has been demonstrated with the help of Log rank test is shown in figure 5.



Graph 4. Kaplan meier survival curve of the different chemicals



Graph 5. Log rank test of survival time (ST) of demodex mite

DISCUSSIONS

Calendula officinalis possesses significant popularity in traditional and cosmetic medicine owing to its anti-inflammatory, analgesic, wound-healing, and antimicrobial characteristics in recent days. Pharmacological components in the plant soothe sore skin, promote healing, and reduce inflammation.[22] Its effectiveness against Demodex folliculorum, a mite linked to many dermatological disorders called demodicosis, is unknown despite its wide range of uses. There is a growing trend toward alternative homeopathic medicine as a result of its affordability, favorable safety profile and assumed compatibility with the immune system.

Previous studies about antimicrobial properties of Calendula officinalis have sufficient evidence on bacteria and fungus related diseases, however its effects on human Demodex folliculorum mites remain unexplored.[23,24] Specifically, we investigated the efficacy of Calendula officinalis tincture in killing Demodex folliculorum and our findings revealed that even at utmost concentration 100% w/v, Demodex mites kept on moving actively for several hours which proved its lack of lethal effect on mite thus demonstrating its therapeutic failure in mite eradication.

Ivermectin, on the other hand still proved to be of highly acaricidal like other previous clinical studies. Ivermectin killed Demodex parasites in an average of 26 minutes as shown in figure 5, thus proving its efficacy without any drug resistance. Lack of therapeutic response of Calendula against Demodex mite in our studies might be due to the lack of powerful bioactive chemicals like terpinen-4-ol, which is present in another herbal tea tree oil, that can strongly penetrate the Demodex mite's chitinous coat and produce mortality effect.[25] Despite its diverse bioactive chemicals like terpenoids, polyphenol, alkaloids, flavonoids, and saponins, calendula cannot penetrate the mite's chitinous protective layer and doesn't produce mortality effect on Demodex mites.[26] Flavonoids and polyphenols of Calendula are more directed towards antioxidant, skin repairing, anti-inflammatory uses and do not typically exhibit the same level of acaricidal activity as other anti-Demodex agents.[27] These compounds confer more skin health benefits rather than for direct mite control. This demonstrated the inferior potency of Calendula when compared to ivermectin as shown in table 2.

One interesting discovery that we observed in our experiment is the presence of multiple Demodex mites on the oily complexioned face of a healthy adult without any visible dermatosis. This contrasts with previous studies suggesting that Demodex proliferation results in demodicosis, which presents with erythematous papule and pustular skin lesion.[28,29] Thus, our findings suggest that only the proliferation of the Demodex mites inhabiting human skin doesn't guarantee demodicosis. The experiment also found that Demodex mites can live in healthy skin without generating any inflammatory skin lesions unless there are significant changes on facial skin pH, skin barrier properties or microbiota homeostasis induced by the skin inflammation, toxin, pollution, poor hygiene, ageing, dysbiosis etc. Thus, demodicosis may require alteration of numerous factors to activate an inflammatory cascade beyond the proliferation of Demodex.

Coconut oil possessing antimicrobial as well as moisturizing nature on stratum corneum via restoring the skin barrier function and balancing the skin microbiota, was once assumed to prevent the occurrence of some variant of demodicosis.[24] But there was no mortality effect of coconut oil on the activities of Demodex mite. Its powerful phytochemical; lauric acid was supposed to have antimicrobial properties mentioned in many studies, however it failed to produce any acaricidal effect on the mites.[30] This demonstrated that combined anti-inflammatory and anti-parasitic therapies must be taken into consideration for controlling as well as eradicating the Demodex-related skin problems.

Calendula officinalis-based skin products might improve facial dermatosis since the anti-inflammatory properties of the plant has been employed in many medical purposes

since ancient times. The phytochemicals possess anti-inflammatory properties thereby supporting its role in reducing inflammation expressed through erythematous papulo-pustular lesion and the irritation in the human skin. The anti-inflammatory mechanism of action of Calendula involves the reduction of pro-inflammatory cytokines, inhibition of nitrous oxide (NO) generation in macrophages, helping to decrease inflammation at both the cellular and systemic levels as well as activating the opioid system and inhibiting COX-2, cyclooxygenase enzyme responsible to producing prostaglandins.[27] This is in accordance with previous studies that Calendula-containing cream can be used as anti-inflammatory topical therapy radiotherapy-induced skin reactions.[31] Such benefits may be attributed to the phytochemicals like terpenoids, phenolic acid and flavonoid compounds present in it. The plant extract did not kill any Demodex mites, thus suggesting that Calendula's medicinal advantages might come from its capacity to reduce inflammatory cascade on the skin rather than eradication of mite population. This skin-related benefit aligns with previous studies indicating its ability to mitigate inflammation in human skin.[32,33] It highlights the skin-rejuvenating, pH-controlling, moisturizing, soothing, and anti-inflammatory potential of Calendula thus maintaining the balanced homeostasis on skin. Hence, Calendula might indirectly regulate the Demodex density on human skin and maintain the parasitostasis in recent trend of prioritizing homeopathic alternative medicines for skin care.

The number of Demodex mites involved in the study might be not sufficient. There might be genetic variation among the mites extracted from patient of different tribe. Variation in age, sex and feeding status of the Demodex mite are excluded from analysis. All these factors aren't considered and compared and might affect the ST and can lead to the limitation of the research study.

CONCLUSION

Although Calendula officinalis has not been established as a parasiticidal agent in our research which was our primary goal, its anti-inflammatory properties from previous studies might remain therapeutically significant in near future. Since skin inflammation is a key factor in disrupting cutaneous homeostasis thereby facilitating the unduly proliferation of Demodex mites this pathological process can be controlled through anti-inflammatory action of Calendula. Therefore, Calendula officinalis may contribute to maintaining parasitostasis by promoting skin rejuvenation while allowing Demodex mites to persist as commensals on the human skin.

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