THE EFFICACY OF CALENDULA OFFICINALIS EXTRACT ON THE MORTALITY OF DEMODEX FOLLICULORUM

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MASTER OF SCIENCE
IN
DERMATOLOGY

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MAE FAH LUANG UNIVERSITY

2024

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THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
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MAE FAH LUANG UNIVERSITY
2024

THESIS APPROVAL

MAE FAH LUANG UNIVERSITY

FOR

MASTER OF SCIENCE IN DERMATOLOGY

Thesis Title: The Efficacy of Calendula Officinalis Extract on the Mortality of Demodex Folliculorum

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ACKNOWLEDGEMENTS

I am profoundly grateful to my respected advisor, Assistant Professor Sirintip Chaichalotornkul, M.D., Ph.D., and my co-advisor, Assistant Professor Tawee Saiwichai, Ph.D. for their tremendous guidance, support, suggestions, and encouragement during my thesis work. Their experience, feedback, and motivation significantly influenced my knowledge, enhanced my experimental endeavors, and improved my scientific contributions in the research field. I sincerely value the time and effort you have dedicated to mentoring me, despite your hectic schedule and significant responsibilities. Your dedication to academic success has been a significant source of inspiration, and I consider myself privileged to have gained insight from you, expecting the same in the years to come. Yours valuable and generous teamwork enabled me to complete the study endeavor within the specified deadline.

I like to express my profound gratitude to Associate Professor Wongdyan Pandii, Dr.P.H. for her precious time and insightful recommendations and feedback, which were important in the completion of this research work. Her thoughtful suggestions are crucial to the understanding of her expertise. I extend my profound gratitude to my family for their encouragement, patience, and consistent backing of my prestigious research projects. Their unending and invaluable support has been a vital source of strength for my academic endeavors. I will always remember the support of my valued participants who contributed to the research effort. I will never forget the support of the staff at Mae Fah Luang University Hospital for their support and facilitation throughout the research period. I also would like to thank Mae Fah Luang University for providing me a partial thesis grant.

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ABSTRACT

Background: A high density of *Demodex folliculorum* along with alteration in skin homeostasis has been associated to *spectrum* of demodicosis. Even though many treatments are available, yet the treatment hasn't been satisfactory and there is growing need of alternatives therapies. The *Calendula officinalis* is thought to have anti-inflammatory and anti-microbial properties, and this hasn't been tested for *Demodex* mite till date. This is the first experimental study to reveal that *Calendula* extract doesn't have direct anti-parasiticidal effect on the *Demodex* mite *in vitro*, but still anti-inflammatory properties of the *Calendula* can help to control the inflammation and thereby maintaining the balanced skin homeostasis. Thus, indirect skin rejuvenating potential of the plant might help to maintain the parasitostasis thus preventing the onset of demodicosis and still allowing *Demodex* to live as commensal on human skin without posing any threat.

Objective: To study the efficacy of *Calendula officinalis* floral extract on the mortality of *Demodex folliculorum.*

Methods: *Demodex* mites collected from patient as hospital waste. A total of 352 slides from patients were collected using skin scraping, squeezing, and Standardized Skin Surface Biopsy (SSSB). They were then exposed to different test agents: including *Calendula Officinalis* extract of different concentration, ivermectin 1%, coconut oil, and immersion oil. Group was randomized by introducing each chemical to a group of 10 mites. Mortality effect was assessed under a microscope based on complete immobility even after needle stimulation. Survival time (ST) from chemical exposure to full immobility was recorded and compared across groups. The experiment was repeated thrice for reliability, ensuring reliable comparison of different chemicals on mite survival.

Results: The *in vitro* experimental study demonstrated that the floral extract of the *Calendula officinalis* didn't have direct parasiticidal effect on the *Demodex* mite even at maximum concentration (100% w/v) and hence wasn't discovered more efficient than ivermectin in killing the *Demodex* mite. Similar effect was observed in case of coconut oil and immersion oil. The efficacy of acaricidal effect on *Demodex* mites follows as: ivermectin 1% > *Calendula officinalis* =coconut oil =immersion oil.

Conclusion: *Calendula officinalis* extract although failed to show parasiticidal effect, its anti-inflammatory potential, can be used to reduce skin inflammation and thus prevent demodicosis. By promoting a balanced skin environment, it helps to prevent excessive *Demodex* mite proliferation. These properties make *Calendula officinalis* a valuable adjunct in inflammation control, indirectly supporting parasitostasis.

Keywords: Acaracides, *Calendula*, *Demodex*, Demodicosis, Efficacy, Mortality, Survival Time (ST)

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ABBREVIATIONS AND SYMBOLS

C1	Concentration
CNS	Central Nervous System
D. folliculorum	*Demodex folliculorum*
EC	Ethical Clearance
HIV	Human Immune Deficiency Virus
IBM	International Business Machine
IPL	Intense Pulse Light
IQR	Interquartile Range
IRB	Institutional Review Board
LMFD	Lupus miliaris disseminatus faciei
MOLT-1	Molecular Oncology Leukemia T-cell
SPSS	Statistical Package for the Social Sciences
SSSB	Standardized Skin Surface Biopsy
ST	Survival Time
T40	Terpinen-4-ol

CHAPTER 1

INTRODUCTION

1.1 Background

Calendula officinalis, a common aromatic herbal plant popularly referred to as English marigold or pot marigold, is an annual plant belonging to the Asteraceae family (Patil et al., 2022). The plant is indigenous to the Mediterranean, Macaronesia, Western Europe, and Southwest Asia but has adapted well to many regions around the world (Ahmed et al., 2023). Gardens and cultivated fields often host the plant, which thrives in temperate climates.

The herbal plant that is most often utilized for medicine and cosmetics widely has been used to treat a variety of diseases since ancient times. *Calendula* 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 eye conditions (Ashwlayan et al., 2018). The anti-tumor and wound-healing qualities of *Calendula* oil are well-known, and its extracts exhibit antiviral and anti-genotoxic properties *in vitro* as well (Ullah et al., 2023).

Calendula is known for its diverse pharmacological therapeutics in the fields of dermatology and cosmetics. Topically, people apply it to heal wounds, reduce inflammation, and soothe irritated tissue. It helps to manage acne by reducing inflammation and controlling bleeding (Chandy, 2023). People also use the plant to treat eczema, as it soothes the skin and reduces irritation. Through tissue repair, *Calendula* oil, known for its wound-healing capabilities, treats skin injuries such as skin damage, scars, frostbite, burns, and ulcer (Rezai et al., 2023). When applied as an infusion, it is beneficial for eye inflammation and bee stings. Clinical tests have shown that cosmetic formulations containing *Calendula* extract cause minimal irritation or sensitization, making them suitable for sensitive skin (Garrido-Suárez et al., 2023). The plant's rich content of active biological ingredients such as flavonoids, saponins,

methanol, and carotenoids contribute to its effectiveness in treating various skin diseases (Shahane et al., 2023).

Demodex mite is an external tiny parasite belonging to the *Demodicidae* family (Mastrota, 2023). It is a common commensal on human skin's pilosebaceous units of the human skin, feeding the sebum and keratin. There are two types of *Demodex* mites found on human skin: *D. folliculorum* inhabits hair follicles, and *D. brevis* resides in sebaceous glands in eyelids. The prevalence of *Demodex folliculorum* colonization in the population may vary between 20% and 80% of the population, and it increases with age (Dopytalska et al., 2019). Normally, such parasites don't cause any harm to human skin, but under certain physiological changes in the skin environment, they can cause dermatoses via mechanical, chemical, and bacterial activities. The excessive growth of *Demodex* mites is more prevalent in patients who are obese, diabetic, immunocompromised, and those with chronic renal failure (Parać et al., 2023). Additionally, topical steroids and immunomodulators may worsen this condition. A high density of *Demodex* mites may cause demodicosis (Paichitrojjana & Chalermchai, 2023), which manifests in many skin conditions such as face dermatitis, eczema, seborrheic dermatitis, folliculitis, rosacea, and acne vulgaris (Paichitrojjana & Paichitrojjana, 2023). Two commonly associated skin diseases are demodicosis and rosacea (Dopytalska et al., 2019). This frequently leads to misdiagnosis. The mites are also involved in skin disorders such as *Pityriasis folliculorum*, perioral dermatitis, pustular folliculitis, and blepharitis (Alex et al., 2023). The association between *Demodex* mites and acne vulgaris is still a subject of debate, since different studies have shown contradictory findings (Thao et al., 2023).

Standard therapies for *Demodex* infestations usually include the use of both topical and oral drugs like metronidazole, permethrin, ivermectin, crotamiton, lindane, and benzyl. Benzoate and some herbal plants decrease the number of parasites as well as associated symptoms. Topical medical treatments often include tea tree oil, which is well-known for its ability to kill mites and its efficacy against *Demodex* mites (Kairey et al., 2023). These treatments also include ivermectin, which directly reduces the number of mites on the skin when administered. Metronidazole, an extensively used topical medication, acts as an antibiotic to control secondary bacterial infections and diminish inflammation. We utilize oral treatments for more severe instances. For this

scenario, ivermectin is used to treat widespread infestations while metronidazole having potent anti-inflammatory and antibacterial properties (Antelo & da Costa Rocha, 2016) is being used to manage both the mites and any accompanying bacterial problems. Together, these therapies have been widely used to control *Demodex* infestations by specifically targeting the mites and reducing the inflammatory reactions they cause (Li et al., 2023).

Despite the widespread availability of treatment therapies, ongoing research continues to revolutionize the treatment outcome of *Demodex*-induced facial dermatoses. As a result of the frequent use of antimicrobial agents leading to drug resistance, associated side effects on the body, and being too costly, there is a diversion of therapeutic intervention towards the use of natural herbal plants (Lam et al., 2018). Considering such shortcomings, researchers have discovered that incorporating herbal plant extracts can lead to synergistic effects, shorten the duration, and lessen the adverse effects of other commonly used parasiticidal agents (Aghazadeh et al., 2023). So, more and more common herbal plants with features like natural friendliness, cost-effectiveness, and fewer side effects are being tested for mite eradication.

Regarding the use of *Calendula* to treat *Demodex* mites, there are several gaps in our understanding. First, most data are informal or gathered from its broad antibacterial capabilities; clinical trials addressing its acaricidal benefits are few. Second, the literature doesn't specifically discuss how *Calendula* could affect *Demodex* mites. Furthermore, there is a lack of data about the best *Calendula* formulations and concentrations for treating *Demodex* infestations. The information needed for the particular concentration modulating the viability of *Demodex* mites at stages of the life cycle remains unexplored in the scientific world.

We still need more research on *Calendula'*s effectiveness in treating *Demodex* infestations. No research has been done so far regarding the effectiveness of *Calendula* on Demodicosis and this has led to some understanding unexplored about the effect of *Calendula* extract on *Demodex* mite. In addition to this, the research gap also comprises the lack of comparing its efficacy with that of established treatments like ivermectin, metronidazole, and tea tree oil, as well as investigating those compounds in *Calendula* that may have acaricidal effects and how they work. Well-designed clinical trials are also necessary to assess *Calendula*'s efficacy in the treatment of *Demodex* mites.

With much evidence-based knowledge lacking regarding the effect of *Calendula* extract on the mortality of Demodex, we would like to test the efficacy of *Calendula* extract on the morality of *Demodex folliculorum* found in many facial dermatoses. Apart from this we also like to study the viability of mites at different points of time and formulate the appropriate concentration of the herbal extract for the therapeutic intervention in common facial dermatoses harboring outgrowth of *Demodex folliculorum*.

1.2 Research Question

Does *Calendula* extract have efficient parasiticidal activity on *Demodex folliculorum* when compared to other treatments?

1.3 Objectives

1.3.1 General Objective

To study the mortality of *Calendula* extract on *Demodex folliculorum in vitro*.

1.3.2 Specific Objectives

1.3.2.1 Primary objective: To evaluate the *in vitro* viability of *Demodex folliculorum* at different concentrations of *Calendula* extract.

1.3.2.2 Secondary objective: To study and formulate the effective dose for *Demodex* treatment by comparing different concentrations (2%, 5%, and 10% and others) of *Calendula on Demodex in vitro*.

1.4 Hypotheses

1.4.1 Does *Calendula officinalis* extract have efficacy in killing the *Demodex folliculorum in vitro*?

1.4.2 Can *Calendula* plant extract change the viability of *Demodex folliculorum* at different lengths of time against the different concentration of *Calendula* extract?

1.5 Conceptual Framework

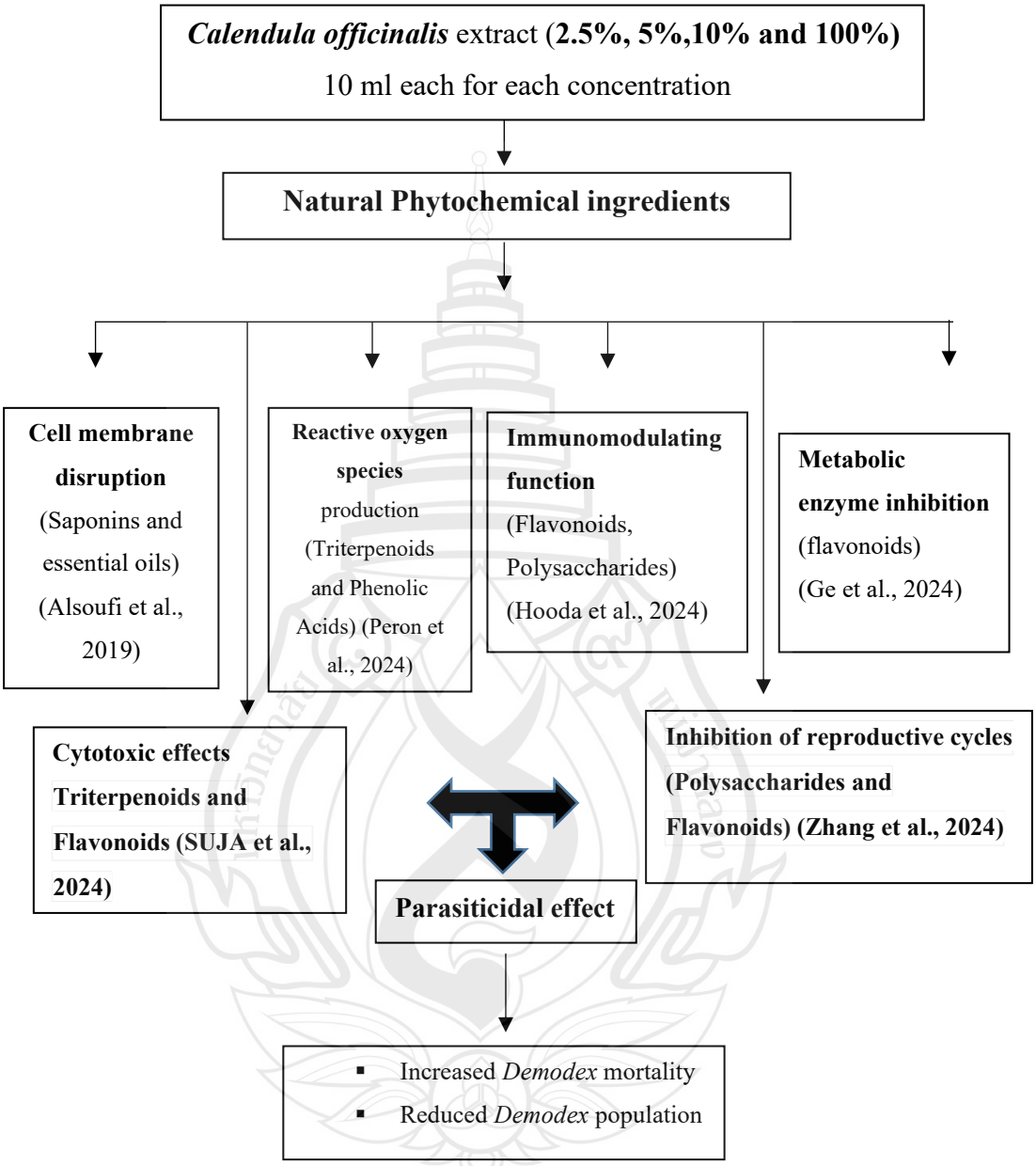


Figure 1.1 Conceptual framework

1.6 Scope of Study

Calendula officinalis may cure *Demodex* mites in the future if scientific study fills information gaps. Improved formulation technology might make *Calendula*-based products suitable for many facial dermatoses particularly for rosacea and blepharitis along with other regular skincare due to its anti-inflammatory, analgesic, and healing potential. *Calendula* may join a dermatological trend toward natural medicines, spurring research and development. Medical standards and consumer awareness might standardize and extend its usage, boosting market growth and new goods in the field of dermatology. *Calendula*'s future in treating *Demodex* mites is bright through scientific evidence-based confirmation and continuous study. Success in these areas could lead to its widespread acceptance and integration into treatment protocols and skincare regimens thereby reflecting a broader trend towards natural and holistic health solutions for the treatment of *Demodex* mite, a common culprit of many facial dermatosis.

1.7 Operational Definition

1.7.1 Blepharitis: Blepharitis refers to inflammation of the eyelid margin. There are two variants based on the location of the inflammation either anterior or posterior.

1.7.2 *Calendula* extract: Chemical herbal extracted from *Calendula officinalis* having widespread therapeutic application.

1.7.3 *Calendula officinalis*: *Calendula officinalis* is a flowering plant from the plant kingdom's Asteraceae family commonly known as marigold.

1.7.4 Coconut oil: A type of oil extracted from the meat of mature coconut.

1.7.5 *Demodex*: A commensal parasite that lives in the follicle and feeds on the sebum and keratin.

1.7.6 Demodicosis: Also known as *Demodex* infestation, is a condition caused by an overpopulation of *Demodex* mites on the skin.

1.7.7 Efficacy: It is the ability to produce the desired result. The efficacy is measured by outcomes like by parasiticidal effect defined by the deaths of the mite on exposure to *Calendula* extract.

1.7.8 Immersion oil: It is a specialized optical oil used primarily in microscopy for visualization under 100X power.

1.7.9 *In-vitro*: It is the experiment in which the samples to be tested have been extracted from the organism.

1.7.10 Ivermectin: A powerful broad-spectrum chemical that is used for killing human and animal mites.

1.7.11 Mortality: Number of deaths in a given period. It is calculated as: Mortality = (total no of deaths/total population) ×10n where n stands for reference number.

1.7.12 *Pityriasis folliculorum: Pityriasis folliculorum* is a form of demodicosis that affects the hair follicle face and causes sandpaper-like texture and irritation.

1.7.13 Rosacea: It's an inflammatory condition of the skin with erythema and telangiectasia.

1.7.14 Survival Time: Interval between the first-time exposure with the test agent and loss of total body movement.

1.7.15 Viability: It is the ability to survive and live successfully.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of *Calendula officinalis*

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asterids

Order: Asterales

Family: Asteraceae

Genus: Calendula

Species: Calendula officinalis (Bhupathyraaj & Amaresh, 2014)

Calendula officinalis is one of the popular herbal plants used for therapeutic purposes in a wide variety of diseases since ancient times in human civilization. It is categorized in the kingdom Plantae, family Asteraceae (Samatadze et al., 2023). Popularly, this plant is known as 'Pot marigold', 'English marigold', Bride of the Sun, bull flower, and butterwort. It is indigenous to the temperate climate of Eurasia and Northern Africa (Hachmi et al., 2023). It is mostly cultivated and can be grown easily in sunny locations in most types of soil. Many gardening experts agree that *Calendula* are among the simplest and most adaptable flowers to grow in a garden area because they can flourish in a variety of soil. Due to the wide diversity in its biological properties, the plants are being cultivated for therapeutic as well as cosmetic and ornamental purposes (Devkota, 2022).

Many parts of the herbal plant including flowers and leaves contain biologically active phytochemicals such as flavonoids, carotenoids, sterols, saponins, phenolic acids, lipids etc. (Dhingra et al., 2022). These substances are used both *in vitro* and *in vivo*. This aromatic *Calendula* is commonly used as an analgesic, antiseptic, diaphoretic, and anti-inflammatory agents because of its predicted therapeutic

properties (Patil et al., 2022). Additionally, the plant extract is also being used in medical issues like burns, gastrointestinal disorders, gynecological problems, dental illnesses, eye illnesses, and skin injuries (Shahane et al., 2023). In its free state, altogether 15 amino acids are discovered in the different parts of the plants like stems, leaves and flowers. One of the most popular parts of the *Calendula* called the flower being processed and different products like extracts, tinctures, and balm were produced for therapeutic purposes in different conditions like skin inflammations (Demodicosis, Acne Rosacea), lacerated and bleeding wounds (Zhang et al., 2020). No known significant medication interactions or contraindications have been noted, although those with a documented significant sensitivity to the community's family may be more vulnerable to adverse reactions.

2.1.1 Physical Description

Calendula officinalis, one of the popular ornamental plant, represents annual or perennial plants possessing somewhat smooth, waxy or glandular stems. The leaves are found to be toothed and they are present alternately along the stems. The leaves of the plant are sessile (lacking a leaf stalk) and may have clasping leaf bases. The composite floral heads consist of orange or yellow coloured ray of flowers and central floral disk that can be red, yellow or purple in appearance (Zhang et al., 2020). The fruit has a curved achene.

Figure 2.1 Morphology *Calendula Officinalis(Ogden)*

2.1.2 Phytochemistry

Numerous research has accurately demonstrated the occurrence of several chemical components found in the plants, with the most encountered being terpenoids, flavonoids, coumarin, quinines, volatile oil, carotenoids, and amino acids. *Calendula*

officinalis, rich in saponins, triterpenoid esters and flavonoids has diversified therapeutics (Dhingra et al., 2022). Due to carotenoid concentration, the blossoms are orange and include amino acids including alanine, arginine, and valine. Other notable chemicals like chloroform extracts, fatty acids, triterpenes, and sterols are also found in *Calendula officinalis* leaves, while its water extract comprises phenolic compounds, saponins, and tannins (Shahane et al., 2023). Ethanolic and aqueous extracts derived from the plant such as alkaloids, flavonoids, and saponins make the *Calendula* useful for therapeutic purposes. *Calendula officinalis* quinone extracts also include plastoquinone, phylloquinone, tocopherol, and ubiquinone, suggesting medicinal potential (Dhingra et al., 2022). Another noteworthy feature is that the orange variety *Calendula officinalis* has more hydrocarbons, while the yellow varieties contain mostly oxygenated derivatives in their phytochemical composition (Shahane et al., 2023).

Table 2.1 Constituents of *Calendula officinalis* flower extract

Main components	Sub-components
Calenduladiol-3-O-palmitate	
Calenduladiol-3-O-myristate	
Oleanolic acid saponins	Calenduloside AH
Oleanane triterpene glycoside	Calendula-glycoside A
Calendula-glycoside A6’-O-n-methyl ester	
Calendula-glycoside A6’-O-n-butyl ester	
Calendula glycoside B	
Calendula glycoside B 6’-O-n-butyl ester	
Calendula glycoside C	
Calendula-glycoside C 6’-O-n-butyl ester	
Calenduloside F6’-O-n-butyl ester	
Calenduloside G6’-O-n-methyl ester	
3-monoesters of taraxastero	
Erythrodiol	
Lupeol	
Brein	

Table 2.1 (continued)

Main components	Sub-components
Ursadio	
Faradiol-3-O-palmitate	
Faradiol- 3-O-myristate	
Faradiol-3-O-laurate	
Arnidiol-3-O-palmitate	
Arnidiol-3-O-myristate	
Arnidiol-3-O-laurate	
Glucosides of oleanolic acid I, II, III, VI, VII	
Glucuronides F, D, D2, C, B and A	
Ester of olanane.	

Source Al-Snafi (2015)

2.1.3 Pharmacological Potential

Calendula officinalis, popularly known as marigold exhibits a wide range of pharmacological effects which include:

2.1.3.1 Antimicrobial and Anthelminthic Effects

Methanol and ethanol extracted from *Calendula* petals demonstrate antibacterial activities against common clinical pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* (Hernández-Díaz et al., 2021).

2.1.3.2 Anti-inflammatory Effects

Preparations from *Calendula officinalis* are widely utilized for wound healing and treating skin inflammation, tissue repair, mucous membranes disease, blister, scars and allergic rashes (Silva et al., 2021). It is effective in treating burn edema and exhibits *in-vitro* inhibition of bacteria, fungi and other microorganisms.

2.1.3.3 Antioxidant and Photoprotective Effects

Calendula officinalis extracts can scavenge hydroxyl and superoxide radicals. Hence plant possesses natural antioxidant properties and confer photoprotective benefits (Bilušić et al., 2024).

2.1.3.4 Cytotoxic Effects

Calendula officinalis inhibits lymphocyte proliferation and has provided some biological characteristics like cytotoxic effects on human lymphocytes without inducing direct mitogenic impacts at the cellular level (Dey et al., 2024).

2.1.3.5 Genotoxic and Antigenotoxic Effects

The floral extract of *Calendula officinalis* inhibits HIV-1 proliferation in acutely infected lymphocytic MOLT-4 cells and is non-mutagenic, nontoxic and non-genotoxic (Jodh et al., 2023).

2.1.3.6 Cardiovascular Effects

Calendula extract reduces the size of myocardial infarctions and may provide cardio-protection by converting ischemic induced death signals into reperfusion survival signals in cardiomyocytes (Jodh et al., 2023).

2.1.3.7 Neuroprotective Effects

Extracts of *Calendula officinalis* exhibit modest sedative effects and can enhance the effects of sedative drugs like barbiturates. They show CNS inhibitory effects and have sedative actions (Ravichandran et al., 2023).

2.1.3.8 Hepatoprotective Effects

Calendula officinalis extracts demonstrate hepatocyte protection against cytotoxicity and oxidative stress produced by toxic carbon tetrachloride (Ullah et al., 2023). They also possess antidiabetic and antihyperlipidemic properties, potentially comparable to insulin (Ullah et al., 2023).

In addition to these pharmacological effects, *Calendula officinalis* has traditional uses for treating internal organ inflammation, gastrointestinal ulcers, dysmenorrhea and convulsions. It is also used as a diuretic, diaphoretic, and detoxifying agent (Venkatesh et al., 2023).

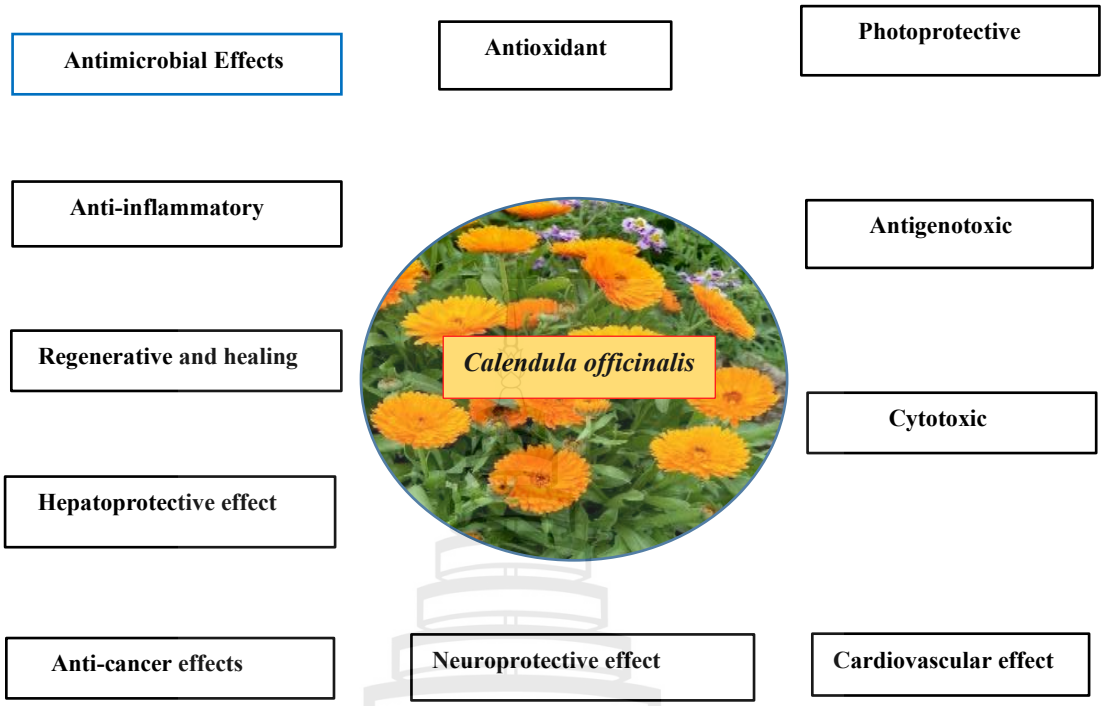


Figure 2.2 Diagrammatic representation of Potential uses of *Calendula officinalis*

2.2 *Demodex folliculorum*

Kingdom: Animalia

Phylum: Arthropoda

Class: Arachnida

Subclass: Acari (mites and ticks)

Order: Prostigmata

Family: Demodicidae

Genus: Demodex

Species: Demodex folliculorum (Prasher et al., 2020)

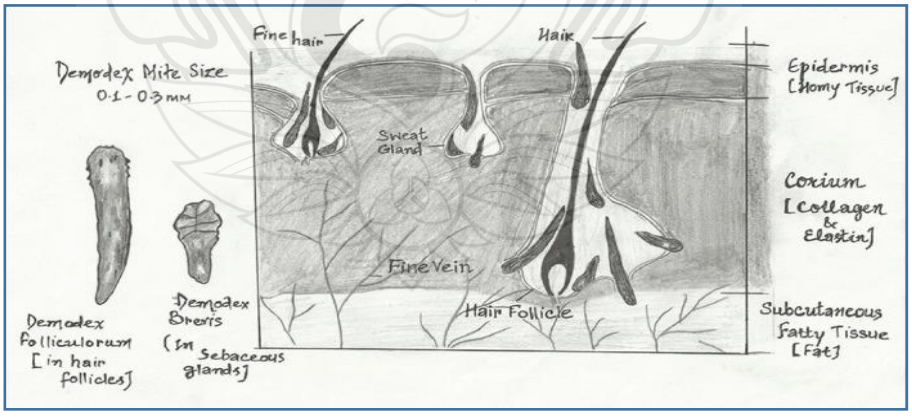
Demodex mites are little ectoparasites arachnids that colonize human hair follicles. They are supposed to be part of the human facial skin fauna, inhabiting within the pilosebaceous unit (Ferhatosmanoğlu et al., 2023). Two main species that have a detrimental effect on people are *Demodex folliculorum* and another *Demodex brevis*. *D. folliculorum* can be observed in the human facial follicular infundibulum, generally in clusters of about ten to fifteen organisms while *D. brevis* is generally found

individually inside sebaceous glands and ducts, mostly on the eyelids, eyelashes and the trunk (Hu et al., 2023). So, such organism is commonly called eyelash mites, face mites or skin mites.

The presence of *Demodex* species on human skin is common and they normally don't pose any threat to humans and rather behave like commensal organisms and feed the sebum and cellular debris. It was found that the *Demodex* mites typically carry some bacteria like *Staphylococcus* and *Bacillus oleronius* bacteria. When the mite density exceeds 5 mites/cm^2, pathogenic over-colonization is predicted to be an indicator of "demodicosis" (Kargadouri et al., 2024). Hence, they behave like both friend and adversary to human beings.

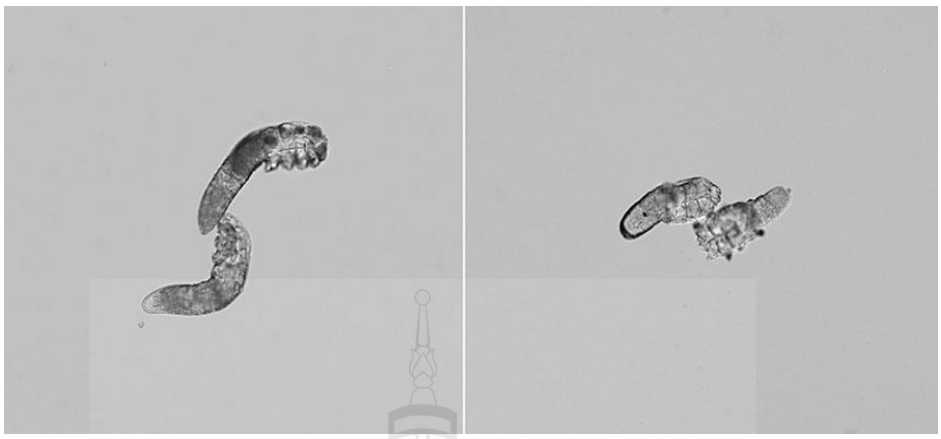
2.2.1 Morphology of *Demodex*

Adult *D. folliculorum* mites are approximately 0.3-0.4 mm long and *D. brevis* are 0.15-0.2 mm long (Kargadouri et al., 2024). Females are shorter and rounder than males. While inconspicuous to the naked eye, their structure is visible under a microscope. Two joined segments represent its semi-transparent and elongated body. Eight short, segmented legs are connected to the first part of the body. This mite moves its eight legs at the rate of 8-16 mm/h at night because intense light necessitates it to retract inside its follicle (Chudzicka-Strugała et al., 2023). The mite contains pin-like mouth parts for sucking up skin cells, hormones, and oils (sebum) in hair follicles and claws and scales for attaching themselves.



Source Rather and Hassan (2014)

Figure 2.3 Morphology and habitat of *Demodex* in the human skin



Source Wei et al. (2024)

Figure 2.4 Microscopic view of *Demodex* mite

2.2.2 Epidemiology of *Demodex*

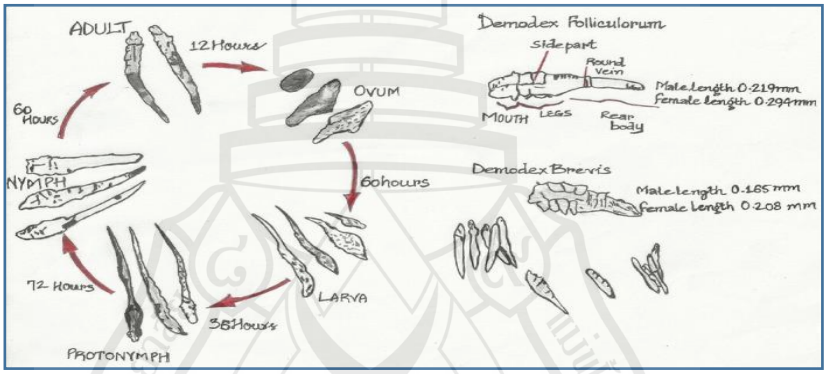
Demodex mites are acquired soon after birth. Since they are situated on the nipple, mother-to-infant transmission presumably begins while feeding. Increasing food supply and sebaceous gland growth during puberty lead to an increased number of parasites in the body. Infection rates have increased with age, peaking in the 5th and 6th decades (Elston & Elston, 2014). *Demodex* infection is common in the aged population and less prevalent in children. They are spread by skin-to-skin contact and are more common among caregivers of the elderly.

Both species may be identified in normal skin biopsies, with *D. folliculorum* being more common and found in higher numbers in individual follicles, particularly on the face. *D. folliculorum* are common on the facial skin, particularly the cheeks, forehead, nasolabial folds and the nose because of their high sebaceous gland density. *Demodex* mites, particularly *D. Brevis* may be detected in the eyelids, ear canals, trunk, and pubis (Tong & Kurji, 2021). Population colonization of men is found to be more common than females in most research studies. This is indeed due to increased androgen-induced sebum production in males. Exogenous lipids in cosmetics may impact *Demodex* mite proliferation.

2.2.3 Life Cycle of *Demodex*

Demodex, is a tiny, microscopic parasite that inhabits in or near hair follicles of the human. *Demodex* females are a little bit smaller and rounder than their male counterparts (Izdebska et al., 2016). A sexual entrance is present in both gender of

Demodex mites and the process of fertilization takes place inside. They come out from the hair follicle on the surface of the skin during the night for copulation. Sexual reproduction takes place at the entrance of the follicle and the laying of eggs takes place inside the sebaceous glands or hair follicles (Rather & Hassan, 2014). The six-legged larvae hatch after three to four days and it takes around seven days for the larvae to mature into adults. The life cycle of this mite is about 3 weeks (14–18/24 days) and the adult mites survive for approximately for a period of one week (Chudzicka-Strugała et al., 2023). For the course of its whole existence, a *Demodex* mite may live for many weeks. Within the pilosebaceous unit, the deceased mites undergo the process of decomposition.



Source Rather and Hassan (2014)

Figure 2.5 Life cycle of *Demodex folliculorum*

2.2.4 Mode of Transmission

The *Demodex* mites are transmitted from the host to healthy people through contact with facial skin, eyebrows and sebaceous glands present on the human skin (Foley et al., 2021). They can also be transmitted through sharing the pillow, towel, mask etc.)

2.2.5 *Demodex*- associated Skin Diseases

Demodex mites have been suggested as the culprits behind persistent inflammatory eruptions of the skin that are like bacterial folliculitis as well as rosacea, perioral dermatitis, and otitis externa. All *Demodex* mite-related cutaneous disorders are referred to as demodicosis (Rather & Hassan, 2014). Even though higher density of *Demodex* mites occur in such settings, no research has provided a conclusive link. In some cases, the treatment with anti-demodectic medication is effective in treating

demodectic alopecia in people, which is a condition that is comparable to animal mangement. Papulopustular rosacea is characterized by several signs and symptoms, many of which are correlated with the frequency of *Demodex* mite infection (Trave et al., 2024).

Most *Demodex* mite carriers do not show any symptoms. Thus, human demodicosis is a multi-factorial illness impacted by environmental and internal causes (Gothe, 1989). Onset of primary and the secondary immunosuppression may be a contributing factor for the transformation of commensal to pathogens thus producing the clinical spectrum called demodicosis (Bøge-Rasmussen et al., 1982; Gothe, 1989). Some of the diseases associated with *Demodex folliculorum* are as follows:

2.2.5.1 *Demodex* – Rosacea

Demodex mites may directly cause rosacea or mimic it as rosacea-like dermatitis, with studies showing higher *Demodex* density in rosacea patients (Ozdemir Cetinkaya et al., 2024). Rosacea-like demodicosis presents as dry skin with exfoliation, vesicles eruptions and pustules formation. Thus, papulopustular variant is mostly associated to higher concentration of *Demodex* mites. *Demodex*-related rosacea can be treated effectively with scabicides like ivermectin, crotamiton, or lindane (Lam et al., 2020) and tea tree oil. Poor facial hygiene and overuse of oily products can enhance lipid nourishment for *Demodex* mites thus promoting their proliferation and causing rosacea-like eruptions.

2.2.5.2 Non-specific facial dermatitis

It has been observed that patients who present with non-specific facial dermatitis like facial pruritus with or without erythema, an eruption similar to seborrheic dermatitis, lesions similar to perioral dermatitis and papulopustular eruption and/or acneiform lesions without visible flushing, comedones or telangiectasia have a marked higher *Demodex* density (Rather & Hassan, 2014).

2.2.5.3 *Demodex*-Acne vulgaris

There is no obvious correlation between high rates of *Demodex* density and prevalence and acneiform eruption like acne vulgaris. However, it is linked to non-specific facial dermatitis and acne especially in those with patchy redness, rough skin, dry, scaly skin, papules that resemble insect bites and flushing (Paichitrojjana & Chalermchai, 2024).

2.2.5.4 *Demodex*- Steroid Rosacea

The role of *D. folliculorum* in the pathogenesis of topical corticosteroid-induced rosacea is controversial. It has been reported that the population of *Demodex* mites is increased in these patients with the excess use of topical corticosteroid preparation (Forton & De Maertelaer, 2021).

2.2.5.5 *Demodex*- Androgenetic Alopecia

The association between *Demodex* and hair thinning in androgenetic alopecia has been mentioned in the literature. It can be concluded that the mite *Demodex* is a factor, not an epiphenomenon of androgenetic alopecia (Millikan, 2001).

2.2.5.6 Madarosis

The infestation of the *Demodex* mite in the pilosebaceous unit of the eyelids can also cause loss of eyelashes (Sławińska et al., 2023). The *Demodex* mite enhance follicular inflammation which triggers edema and easier eyelash epilation. It also affects the constriction of the cilia, subsequently rendering the lashes brittle and falling off.

2.2.5.7 Lupus Miliaris Disseminatus Faciei (LMDF)

Many authors have suggested that LMDF is an endogenous reaction to *D. folliculorum*; however, a definite association has not been confirmed yet (Rather & Hassan, 2014).

2.2.5.8 Scalp Dissecting Folliculitis

It is unclear what causes scalp dissecting folliculitis. The prevailing consensus is that it is an inflammatory response to the contents of the hair follicle, namely microorganisms such as bacteria (particularly *Propionibacterium acnes* and *Staphylococcus aureus*) and yeasts (Rather & Hassan, 2014).

2.2.5.9 Other associated conditions

A higher population of *Demodex* mites has been discovered in peri-oral dermatitis, acarica blepharo-conjunctivitis, Grover's disease, papulopustular facial dermatitis, eosinophilic folliculitis, papulopustular scalp eruptions, *Pityriasis folliculorum*, pustular folliculitis, *Demodex* abscess, and demodicosis gravis (granulomatous rosacea like demodicosis (Li et al., 2021; Rather & Hassan, 2014).

2.2.6 Treatment of *Demodex* Mite

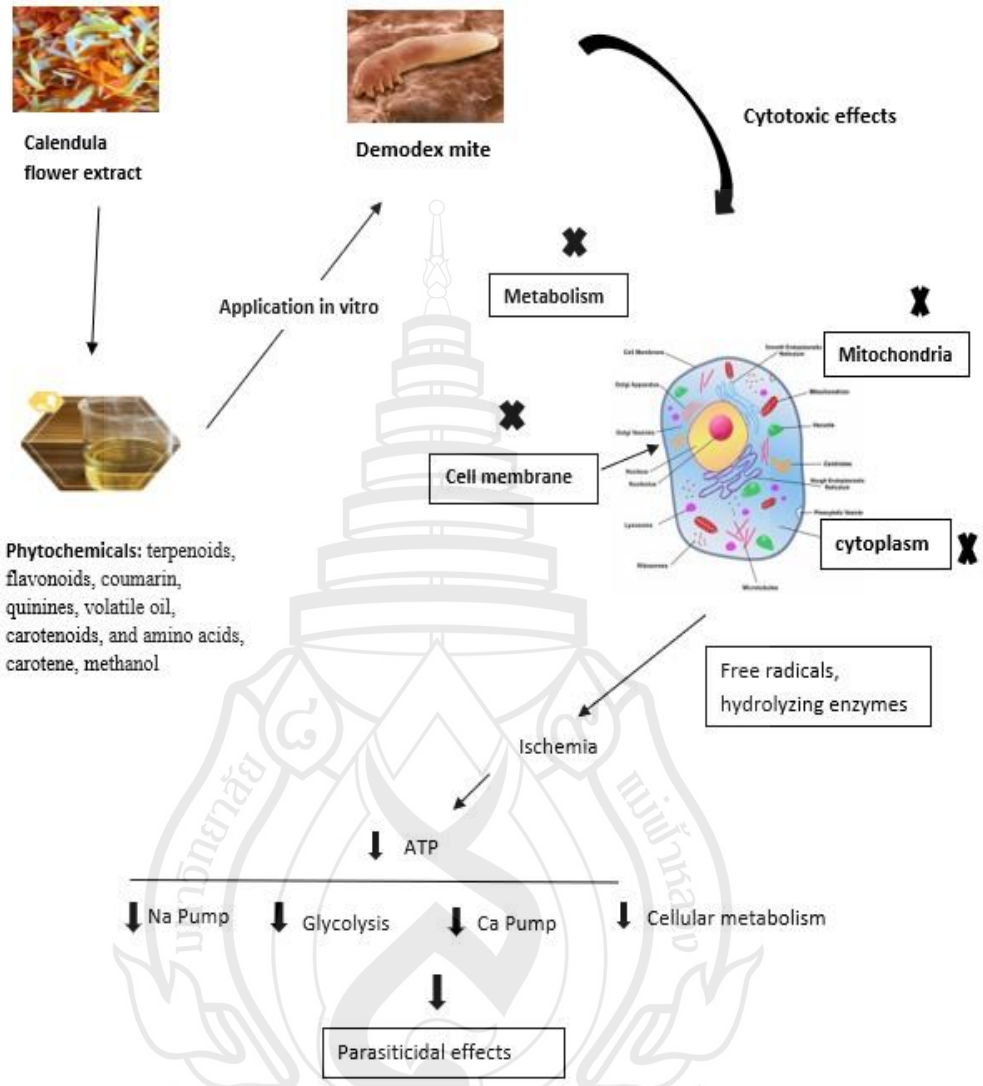


Figure 2.6 Mechanism of action of *Calendula* extract on *Demodex* mite

There are many treatments available for the management of ectoparasite *Demodex* residing in the human skin. The currently available treatment includes topical applications like metronidazole, ivermectin, permethrin, crotamitron, sulfur-based ointments, tea tree oil, etc., and oral medication like ivermectin and metronidazole. These pharmaceutical agents eliminate the organism from human skin through anti-inflammatory and cytocidal effects thereby conferring anti-parasiticidal effects.

The efficacy of these drugs has been compared in different studies in the research. In one study conducted comparing the efficacy and tolerability of the sulfur-

sodium sulfacetamide combination, crotamiton, and permethrin, it was found that there is no statistically significant difference in terms of patient satisfaction and clinical evaluation (Sarac, 2019). The selenium sulfide was found to be more efficacious in producing lethal effects on *Demodex* at a 4% concentration with the CMC solution and mild activity with 4% petroleum jelly (Heczko et al., 2023). In one study conducted by two researchers Paichitrojjana and Chalermchai about *in vitro* killing efficacy on *D. folliculorum* it was found that herbal-based products and tea tree oil were found more efficacious than 1% ivermectin and metronidazole 0.75% (Paichitrojjana & Chalermchai, 2023). Apart from this, metronidazole was found to be less efficacious than ivermectin. Due to the associated side effects such as ocular irritation, the researcher advised using use lower concentration of tea tree oil for *Demodex* blepharitis (Savla et al., 2020). Such therapies have also been combined with laser light therapies, especially Intense pulse light (IPL) and skin cleansing procedures for the effective control of *Demodex* mite infestation.

Till now no comparative studies have been done about the *Calendula* extract with other conventional therapies regarding the parasiticidal effect on *Demodex*. Since other common medications have been associated with drug resistance and adverse drug reactions, there has been a growing need for the newer treatment which could be more efficient in killing. Apart from this the drugs are costlier and pose more financial burdens to the patient thus directing the alternative treatment modalities.

Since the *Calendula* plant is easily available plants around the globe and cultivated for aromatic, ornamental, and medicinal purposes the phytochemical extract from such plants can be an alternative treatment for the ectoparasite. Being rich in diversified phytochemicals the *Calendula* possesses antioxidant, anti-inflammatory, antimicrobial, and healing properties the plant extract can be mobilized in the treatment of many dermatoses associated with *Demodex folliculorum* mite.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

This study was a Laboratory-based Experimental Study to assess the efficacy of *Calendula officinalis* on the mortality of *Demodex folliculorum.*

In addition to this, the study also focused on testing viability of the *Demodex folliculorum* at different points of time with the use of different concentrations of the floral extract of *Calendula officinalis*.

3.2 Study Population

Demodex folliculorum (waste from the hospital).

3.3 Sample Size

For this, we made a group of 10 mites for each experimental agent (chemicals) and operated the research similar to the one that has been done before (Paichitrojjana & Chalermchai, 2023).

3.4 Sample Size Determination

For this we categorized a group of 10 mites for each experimental agent (chemicals) and operated the research similar to the one that has been done before (Paichitrojjana & Chalermchai, 2023). A group of 10 mites belonging to particular group were exposed to different testing experimental chemicals and the procedure was similar to that of previous experiment (Paichitrojjana & Chalermchai, 2023).

3.5 Inclusion Criteria

3.5.1 Live mites (mites with active movement).

3.5.2 Mites extracted from facial dermatoses.

3.5.3 Adult form of Demodex folliculorum

3.6 Exclusion Criteria

3.6.1 Mites with no body movement

3.6.2 Mites with injured body parts

3.6.3 Mites with damaged legs during extraction and preparation of the slides.

3.7 Discontinuation Criteria

3.7.1 Mites showing no life during the preparation of the slides.

3.7.2 Mites that have damaged body parts during the experiment period.

3.7.3 Mite who has been accidentally exposed to excess testing chemical agents.

3.7.4 Accidental exposure of mites to unrelated chemical/testing agents.

3.8 Study Location

Mae Fah Luang University Hospital, Bangkok

38/11-13 Asoke Place, Sukhumvit 21 Road, Khlong Tan Nuea, Watthana District, Bangkok, 10110. Tel. 02-664-2295

3.9 Variables of the Study

Independent variables of the study included 1% ivermectin, immersion oil, coconut oil and 3 different concentrations of *Calendula officinalis* extract.

Similarly, the mortality effect of *Demodex* mite and viability time/survival time at different periods behaved as dependent variables for the research study.

3.10 Research Materials and Methods

3.10.1 Research Materials

All the chemical required for the research study was obtained from different registered manufacturing companies with valid manufacturing date. *Calendula officinalis* tincture (100ml) 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, coconut 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 (Nikon Eclipse E100, Japan).

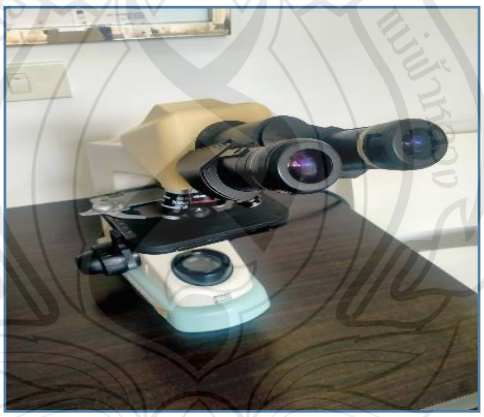


Figure 3.1 Microscope (Nikon Eclipse E100, Japan)

3.10.2 Research Methods

Testing the parasiticidal effects of a chemical extract derived from the flower of *Calendula* against the mortality effect of *Demodex* mites in a laboratory involved several methods and steps. These procedures focused on the effectiveness of the efficacy of a given chemical agent in killing the mites which was confirmed by the Nikon microscope.

The research methods used in such testing involved:

3.10.2.1 Collection and Preparation of the Adult Healthy *Demodex* Mites:

1. Source: *Demodex* mites were typically collected from hospital waste of the patient suffering from facial dermatoses like Acne, Rosacea, *Demodex* folliculitis, etc. Altogether, a total of 352 slides were obtained from 44 patients using various procedures such as squeezing, skin scraping, and Standardized Skin Surface Biopsy (SSSB).

2. Identification: Healthy motile mites were then identified under a microscope to make sure that they were indeed *Demodex* mites.

3. Transfer of mite: After the extraction from patients, the mites were transferred to laboratory immediately and the testing chemicals were introduced with the help of pipette/dropper.

3.10.2.2. Preparation of Chemical Solution:

1. Chemical Agent: The different chemicals being tested such as *Calendula* extract (prepared from the floral parts), ivermectin, coconut oil, emersion oil, etc. were prepared in various concentrations for the experiment. Desired final volume was taken approximately 10µl solution containing 0.1 mg ivermectin for 1% ivermectin. For rough estimation 10-15 ml gel solution was enough for testing 10 mites in the laboratory. Similarly different concentration of *Calendula* extract was prepared by using the formula: $C_1V_1=C_2V_2$ (where C_1 and V_1 refers to initial concentration and volume of solution where C_2 and V_2 refers to final concentration and volume of the solution).

2. Solvents: Appropriate solvents needed for the solution was taken ensuring that the solvent didn't have a toxic effect on the *Demodex* mites.

3.10.2.3 Exposure of Mites to Chemicals

1. Direct Application *in vitro* Methods: *Demodex* mites were placed on a glass slide. Then the correct and labelled prepared chemical solution of six samples was introduced slowly to them by a dropper/pipette.

2. Immersion: Mites were immersed in the prepared chemical solution for six hours.

3.10.2.4 Observation and Counting of Mites:

Microscopic Examination: *Demodex* mites were observed under a microscope at 10X and 40X at various time intervals post-exposure to assess viability and mortality.

1. Viability Criteria: Mites were considered dead if they exhibited no movement or response to stimuli (e.g., gentle pressing with a needle).

2. Control Groups: Parallelly, the experiment was conducted with positive control (ivermectin solution) and negative control (immersion oil) to compare the efficacy of *Calendula* extract.

3.10.2.5 Data Collection and Analysis

1. Mortality Rate: The number and percentage of dead mites were recorded and calculated at each time point. The concentration and its effect on mortality was calculated and recorded in a form.

2. Statistical Analysis: Statistical analysis was done by SPSS software-21 in a computer and the mortality effect on *Demodex* among the different test chemicals were expressed in the form of Log- rank test and the Kaplan Meyer curve.

3.11 Research Procedure

3.11.1 10 mites were randomly selected for each group of test agents.

3.11.2 Ivermectin solution 1% was assigned as positive control and immersion oil as negative control for the experiment.

3.11.3 A total of six chemical testing agents behaved as six groups (ivermectin, immersion oil, chemical gel, 2.5% *Calendula* extract, 5% *Calendula* extract, 10% *Calendula* extract) and other concentration (100% as well) as needed which was prepared from floral parts.

3.11.4 Each group was tested on 10 mites for 3 times to make more reliable studies.

3.11.5 Microscopic evaluation was carried out after being exposed to testing chemicals.

3.11.6 The viability of mites was observed carefully for a total period of 6 hours.

3.11.7 Survival of the mite was determined by testing the motility at different periods of time.

3.11.8 For the 1st hour, the viability was tested every 2 mins over 1 min and for the 2nd hour, the viability was tested every 10 mins. Similarly, during 3rd hour this process was done every 20 minutes, and at the last hour, the viability was tested every 30 minutes.

3.11.9 The death of the mite signified the loss of viability.

3.11.10 The death of the mite was considered when there was no movement of head, body, and leg over 2 mins even when pressed by the needle on slides.

3.11.11 The total survival time of the mite was noted and recorded in a form.

3.11.12 The independent variables in this study were *Calendula officinalis* extract (at different concentrations 2.5%,5%, 10% and 100%), ivermectin 1%, coconut oil and immersion oil. The dependent variable was Survival Time (ST), which measured the time to kill the *Demodex* mites after exposure. Statistical tests like Log Rank Test and Kaplan Meyer Curve was used using IBMS SPSS 21 to assess the relationship between the treatment and mortality time.

3.12 Bias and Bias Control

The expected bias during the experiment was controlled with randomization of *Demodex* mites into six or more groups and each group of *Demodex* mite group in the petri dish/glass slide will be assigned unique code (E.g., A, B, C, D, E, F, G, H….) and the death of the mites under microscope was identified separately by two different lab staff/doctors.

Another factor that that could cause errors in the experimental outcome was time and intensity of light exposure. So, this was reduced by doing experiment at the same time of the day and exposing the light for equal period during the observation of mites under microscope light (like 2 minutes for all group of mites). Another expected bias was due to unequal volume of the testing chemical. So, it was controlled by taking equal amount of testing chemicals which was measured by the syringe/dropper. Similarly, bias regarding variability in mortality time was minimized by observing the

mites under microscope in equal interval of time for all group of mites with the help of timer.

3.13 Data Collection

The data was collected about the testing agents and their concentration, the time of application of the testing agents, and the viability of mites at different periods. Additionally, the number of mites died at particular time was recorded in the record form and counting of mites was done at different interval over a period experimental time.

3.14 Statistical Analysis

Documentation of the medical records of the experimental data and the outcomes of the experiments in this study were recorded with the help of Microsoft Excel 2022 and SPSS Statistics version 21.0 (IBM Corporation Armonk, NY, USA).

3.14.1 Descriptive Statistics

The data collected were analyzed and interpreted by different methods like count, percentage, median, mean mode, interquartile range, Standard Deviation, etc.

3.14.2 Inferential Statistics

The data calculated among the group were analyzed statistically by different tests for comparing mortality time for 6 groups. The time to event data were expressed in the form of Kaplan-Meier curve**.** The comparison of survival time (ST) between different groups was analyzed by a Log Rank test.

3.15 Ethical Consideration

The ethical approval was taken from the IRB (Institutional Review Board) committee of Mae Fah Luang University, Chaing Rai, Thailand. Since the sample for the experiment consist of the *Demodex folliculorum* mites obtained from hospital waste

(ie sebum, exfoliated skin) the letter of exemption was obtained for initiating the research project having the EC protocol number 24149-20 on 5th September 2024.

CHAPTER 4

RESULTS

The research study was conducted to test whether the floral extract of the *Calendula officinalis* is efficient in killing the *Demodex folliculorum* (face mite) in vitro or not. Additionally, this is also focused on the comparative study of parasiticidal effects of different chemicals like *Calendula officinalis* and coconut oil versus Ivermectin 1% solution on *Demodex folliculorum* extracted from the human face.

The efficacy of the *Calendula officinalis* on killing *Demodex* mite and comparative studies were evaluated in terms of Survival time (ST) that represented the time taken to kill the *Demode* mite from the time of chemical application to the cessation of movements of the body parts and limbs which was independently evaluated by 2 doctors and laboratory staffs.

The research results were presented as follows.

1. General characteristics of subjects
2. Mean Median and Range of the ST of different chemicals.
3. Log Rank Test for Efficacy of Treatment on *Demodex*
4. Kaplan-Meier curve for time to event data representation:

4.1 General Characteristics of Subjects

The research study comprised of total 44 participants at different time of 4 months, at Mae Fah Luang University Hospital, Bangkok, from December 2024 to March 2025. None of the volunteers discontinued during the *Demodex* extraction in the OPD. The demographic data of all volunteers taking part in 1–3-day hospital visit is demonstrated in Table 4.1 The study involved 15 female and 29 male participants from the total 44 participants either temporarily or permanently all residing in Bangkok city, Thailand. Most of the participants were among the age group of 30-40 years which included total of 25 people willing to participate in the research studies.

Among the participants enrolled in the study, 16 people were found to have facial dermatosis that comprises 36.3% while remaining 63.4% consisting of 28 participants were devoid of any form of facial dermatosis. All the people didn't use any medication in the previous 2 months. Their occupations included three office workers, one student, and fifteen government officers, fifteen restaurant staff and ten participants work in others shop as shopkeeper.

According to the Fitzpatrick scale, skin type classification identified total of twenty-nine participants as type-IV, ten as type -III, and five as type-V. The predominant skin condition on our research study was oily, which was reported in thirty-five subjects, followed by combination skin in five participants and dry skin in four of the participants. None of the participant had any known underlying diseases or allergy to chemicals and none of them developed allergy to acrylate that was used for taking skin biopsy.

Table 4.1 Gender demography

Gender	Frequency	Percent %
Male	29	65.9
Female	15	34.1
Total	**44**	**100.0**

Table 4.2 Fitzpatrick skin type of the participant

Age in years	Frequency	Percent (%)
20-30 years old	5	11.4
30-40 years old	25	56.8
40-50 years old	8	18.2
50-60 years old	6	13.6
Total	**44**	**100.0**

Table 4.3 Age of the patient

Fitzpatrick Skin Type	Frequency	Percent (%)
Fitzpatrick Skin Type-III	10	22.7
Fitzpatrick Skin Type-IV	29	65.9
Fitzpatrick Skin Type-V	5	11.4
Total	44	100.0

Table 4.4 Facial dermatosis

Disease status of face	Frequency	Percent (%)
Patient with Facial dermatosis	16	36.4
Patient without facial dermatosis	28	63.6
Total	**44**	**100.0**

4.2 Mean Median and Range of the ST of Different Chemicals

Table 4.5 Descriptives table for Survival time (ST)

	n	Mean	Std. Deviation SD	Minimum	Maximum
Calendula officinalis	30	360.00	.000	360	360
Ivermectin 1%	30	25.47	7.295	18	42
Immersion oil	30	360.00	.000	360	360
Coconut oil	30	360.00	.000	360	360
Total	**120**	**304.24**	**125.055**	**18**	**360**

Table 4.5 presents comprehensive descriptive data regarding the chemical evaluated for mite mortality. As the positive control, ivermectin killed the most mites, with a mean duration of 25.47 minutes and a standard deviation of 7.29 minutes. From the four chemicals that were tested, this was the most effective. The mites exhibited varying mortality over different time intervals, ranging from a minimum of 18 minutes to a maximum of 42 minutes. It was found that the extract of *Calendula officinalis* did not kill *Demodex* mites at any concentration. This meant that the mites were still alive after 360 minutes, or 6 hours, and were then killed. In the same way, immersion oil (used as a negative control) and coconut oil for *Calendula* had no effect on the death of *Demodex* mites in the lab. The survival period for *Calendula*, immersion oil, and coconut oil was censored, and no impact on *Demodex* mortality was seen. As a result, the table revealed no mean, standard deviation, or range.

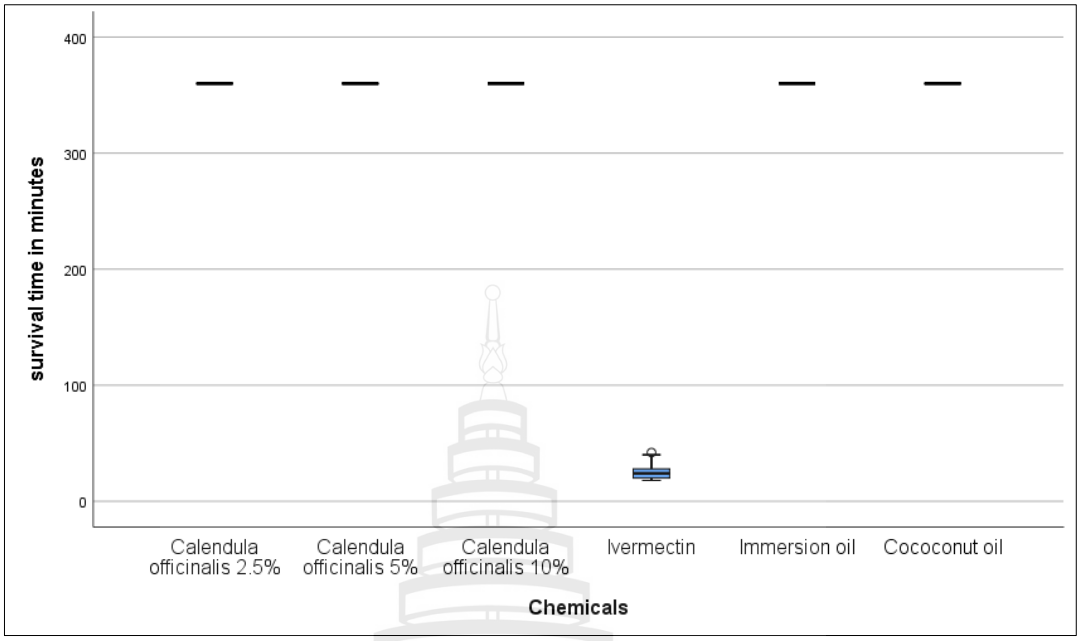


Figure 4.1 Boxplot (IQR) for Survival Time

The boxplot (interquartile range graph) compares the survival period (in minutes) of mites subjected to various substances. Different concentrations of *Calendula officinalis* demonstrated survival, with mites surviving throughout the entire research period. Ivermectin 1% exhibited the shortest survival durations, indicating increased mortality (early fatalities). Immersion oil and coconut oil had modest survival durations, with diversity observed among the treated mites.

Table 4.6 Case processing summary

Chemicals	Total	Number of Events	Censored		
	n		n	1=dead & 0=alive	Percent
Calendula officinalis 2.5%	30	0	30	0	100.0%
Calendula officinalis 5%	30	0	30	0	100.0%
Calendula officinalis 10%	30	0	30	0	100.0%

Table 4.6 (continued)

Chemicals	Total n	Number of Events	Censored		
			n	1=dead & 0=alive	Percent
Ivermectin 1%	30	30	0	1	0.0%
Immersion oil	30	0	30	0	100.0%
Coconut oil	30	0	30	0	100.0%
Overall	180	30	150	0	83.3%

Table 4.6 explains the case processing summary. Among all four tested chemicals on the mortality of *Demodex* only the 1% ivermectin showed mortality events thus indicating the parasiticidal effect on mite. While the *Calendula officinalis* didn't show any effect on the *Demodex* mites, and the mites remained alive even after full period of experiment i.e. 360 minutes. Similar effect was also observed on coconut and immersion oil. The events during the experiment were censored (1=death and 0=alive) since mites didn't die even after 6-hour period.

4.3 Log Rank Test for Efficacy of Treatment on *Demodex*

Table 4.7 Log rank test for comparing the median survival time (ST) differences between *Calendula officinalis* floral extract, positive control (ivermectin 1%), and negative control (immersion oil) and coconut oil

No	Test Agents: Chemicals	P-value
1	Ivermectin 1% (Positive control) versus (immersion oil) negative control	<0.05
2	*Calendula officinalis* (Diff conc) versus (ivermectin 1%) positive control	<0.05
3	*Calendula officinalis* (Diff conc) versus (immersion oil) negative control	1.00
4	*Calendula officinalis* (Diff conc) versus coconut oil	1.00
5	(Ivermectin 1%) positive control versus Coconut oil	<0.05
6	Coconut oil (Diff conc) versus (immersion oil) negative control	1.00

Table 4.7 There were marked differences in how long the *Demodex* mite survived when they were exposed to Ivermectin 1% and immersion oil, *Calendula officinalis* (different concentrations) and coconut oil. No significant differences were observed among *Calendula officinalis*, immersion oil, and coconut oil.This means that ivermectin 1% is more efficient in killing mites than the other test chemicals in most tests, though *Calendula officinalis* and coconut oil have results that are about the same as immersion oil.

4.4 Kaplan-Meier Curve for Time to Event Data Representation

The survival time of the different testing chemicals were represented by the Kaplan-Meier survival curve. Y axis represented the cumulative survival percentage while horizntal x axis denotes the survival time measured in minutes for 6 hours.

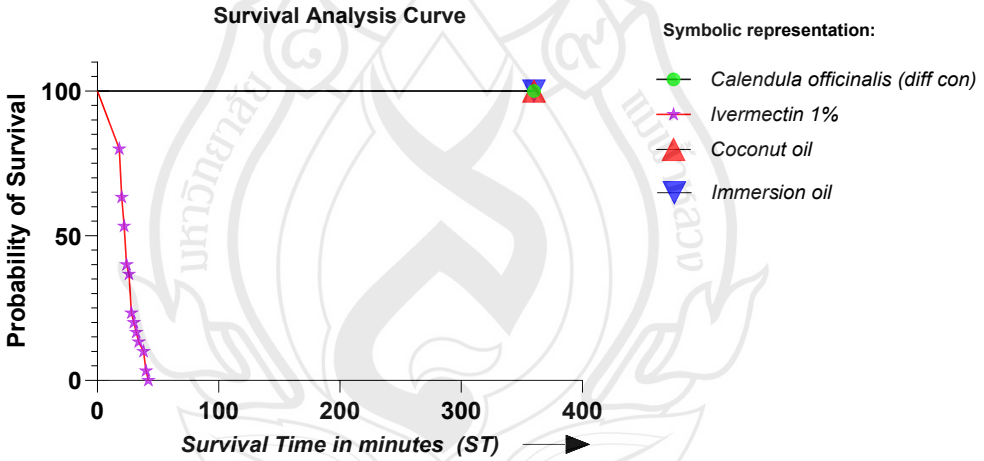


Figure 4.2 Kaplan Mayer Curve

The Kaplan-Meier survival graph above illustrated the effect of several chemicals on the survival of *Demodex folliculorum* mites over a duration of six hours. The Y-axis in the picture indicates the likelihood of survival probability, beginning at 100%, while the X-axis depicted survival time in minutes over 360 minutes. Initially, all the mites were alive, resulting 100% probability of survival as represented on the y-axis. Observation was focused on how long the mites survived after the introduction of different chemicals, including *Calendula officinalis*, ivermectin, and immersion oil and

coconut oil. Ivermectin 1% demonstrated the most efficacy in killing the mites, as evidenced by its survival curve, within thirty minutes. The presence of mite marked along the curves indicated that data was suppressed, suggesting that mites persisted until the end of the observation period. The horizontal line at the top, aligned with the x-axis, indicated the mite remained still alive even after observing for 6 hours with *Calendula*, immersion oil and coconut oil on *Demodex* mites. This diagram indicated that ivermectin 1% had a powerful parasiticidal effect on *Demodex*, whereas no effect is detected with the other three drugs (*Calendula*, coconut oil and immersion oil) even at maximum 100% over a period of 6 hours.

CHAPTER 5

DISCUSSION

Calendula officinalis is a common herbal plant, considered to have originated in the southwest Mediterranean region that has been in use for a variety of purposes in our daily lives since ancient times (Goncalves et al., 2018). Researchers have discovered numerous properties including its anti-inflammatory (Della Loggia et al., 1994), analgesic, aroma, wound healing (Rezai et al., 2023), and antibacterial properties. Cosmetics and dermatology have made extensive use of *Calendula* plant extract (Lohani et al., 2021). The plant has been used to treat several dermatological conditions because of its strong pharmacological components, which lessen irritation, soothe damaged skin, and slow down the inflammatory process (Kiaei et al., 2018).

A common microorganism on the human skin *Demodex folliculorum* behaves as a foe and friend (Foley et al., 2021; Paichitrojjana, 2022). A high density of *Demodex folliculorum* has been linked to several skin diseases collectively referred to as demodicosis (Paichitrojjana & Chalermchai, 2023). Despite numerous treatments for *Demodex* mites, results remain unsatisfactory. The high cost and side effects of the treatments have contributed to this issue. Even some *Demodex* mites are found to elicit resistant to ivermectin which has led to use of other alternative drug (Paichitrojjana & Paichitrojjana, 2022). This concern has led to ongoing research into better and alternative treatment modalities that could improve the efficacy of anti-*Demodex* treatment medication. Bio-ingredients and phytochemicals found in the plant extract of *Calendula officinalis* have been shown to have antimicrobial properties (Efstratiou et al., 2012), so it was investigated to observe how well it bring acaricidal effect on *Demodex* mites that were taken from the participants. The mortality of *Demodex folliculorum*, which is found in human skin responsible for many diseases was the subject of the experimental investigation.

According to our research, there is no mortality effect on the active, motile, and healthy *Demodex* mites even after application of the *Calendula officinalis* tincture several times. Even at the maximum concentration of *Calendula* tincture, 100% w/v,

the mites moved actively on the glass slides thus proving no reaction to *Calendula* tincture. Additionally, all the mites continued to crawl actively even beyond the anticipated six-hour testing period. When compared to mortality effect of another herbal plant in the literature ie. tea tree oil, the tea tree oil was more efficient than any other agent in spite being herbal plant. The efficient effect could be due to the powerful chemical terpinene-4-ol that is strong poisonous molecule that can pierce thick chitin cuticles of *Demodex* and brought cytotoxic effects. This acaricidal molecule present in herbal plants like tea tree oil has produced lethal effect in *Demodex* mites within few minutes of application (Bezabh et al., 2022; Paichitrojjana & Chalermchai, 2023). The absence of such chemical in *Calendula officinalis* could be one of the strong reasons for the failure to produce mortality effect. Additionally, another factor might be due to insufficient dose ie higher dose than that used in the experiment might be required for the *Demodex* killing.

Calendula officinalis contains various phytochemical having diverse pharmacological potential such as alkaloids, terpenoid, coumarin, flavonoids, saponins, and numerous other antioxidants (Rigane et al., 2013; Zournatzis et al., 2025). Researchers have discovered that these phytochemicals possess antimicrobial properties against specific microorganism like bacteria, virus and helminths (Efstratiou et al., 2012; Godara et al., 2015; Kalvatchev et al., 1997; Zournatzis et al., 2025). In contrast to tea tree oil, which is another herbal plant extract, patient can get rid of the facial *Demodex* mite because it contains terpinene-4-ol, a highly poisonous compound that breaks down *Demodex*'s cell wall and kills it in 10 minutes (Paichitrojjana & Chalermchai, 2023). It demonstrated that the phytochemicals in *Calendula* are insufficient to break down the lipophilic layer of the *Demodex* mite and produce cytotoxic effects.

Our analysis also revealed the atypical presence of nearly two dozen of *Demodex folliculorum* mites on the face of a healthy adult without any known facial dermatosis. The patient had an oily complexion that promoted the existence of *Demodex*, thereby balancing the production and consumption of sebum. This evidence revealed that only the proliferation of *Demodex folliculorum* mites does not necessarily result in demodicosis. The *Demodex* mite needs to be in balance with several factors that control physiological processes needed for healthy skin growth and turnover. Skin

inflammation is one of the important contributory factors for disrupting the skin homeostasis that provides platform for the *Demodex* proliferation. Anti-inflammatory potential of *Calendula* can be mobilized to keep the skin environment in balanced state thus keeping the mite population in controlled state. This anti-inflammatory property of *Calendula* provides parasitostasis effect on the human skin thereby preventing the onset of demodicosis.

Ivermectin, another commonly used parasiticidal medicine continued to exhibit lethal effects on *Demodex* mites as in previous studies (Demirci, 2023). The drug works by attaching to gamma-aminobutyric acid-gated chloride ion channels in neuronal synapses thus producing mortality on *Demodex*. The medicine produced mortality effect on all mites within thirty minutes which is almost similar to that in previous studies. Some form of ivermectin refractory demodicosis has also been discovered (Paichitrojjana & Paichitrojjana, 2022). In comparison to the prior study, the *Demodex* treated with ivermectin were eradicated in an average of 26 minutes, rather than the previous 17 minutes throughout the experiment. This evidence further still revealed the powerful acaricidal properties of ivermectin, even at a low dosage of 1%. The different survival duration of the *Demodex* mite may be influenced by its eating state, age, and genetic composition.

The other two chemicals, coconut oil and immersion oil, also have no effect on mortality since they are unable to kill the active, motile *Demodex* mites. Even after six hours of therapy, coconut oil, which was once thought to possess antimicrobial properties because of 50% lauric acid, demonstrated no effect on the mortality. Despite coconut oil's modest antimicrobial properties (Nasir et al., 2018), the study showed that *Demodex* mites can avoid its antimicrobial effects. However, it has been discovered that the coconut oil might still contribute in restoring the compromised skin barrier and disbalanced microbiota on human skin thus preventing demodicosis. One noteworthy significant finding in our study is that having many *Demodex* mites on the face—even 18–20 mites—doesn't necessarily indicate demodicosis since this patient didn't complaint any facial dermatosis. Numerous variables that regulate the skin's physiology may influence the development of demodicosis. This result generalized the idea that anti-inflammatory drugs should also be considered to control the diseases along with anti-parasitic drugs since inflammation seen in many skin disease is one of

the important contributory factor leading to the change in skin ph, moisturizing factor alteration, skin microbiota disturbances etc. This provide favorable environment for the proliferation of *Demodex* mite and causing human skin diseases. From the result of our study the idea was generated that maintaining the skin homeostasis is utmost necessary for balancing the mite population on the human skin and this can be brought by the anti-inflammatory nature of *Calendula officinalis* and thus maintaining the parasitostasis state of the human *Demodex* mite.

The anti-inflammatory properties of the phytochemicals present in *Calendula officinalis* are well-supported by prior research, which highlights their capacity to attenuate the inflammatory cascade. This modulation of inflammation may contribute to maintaining the physiological equilibrium of the skin, thereby helping in balancing *Demodex folliculorum* population on the facial skin. Given that demodicosis is influenced by multiple cutaneous factors, the ability of *Calendula officinalis* to reduce localized inflammation provides a more stable and balanced cutaneous environment. Consequently, it may contribute indirectly for maintaining parasitostasis by fostering conditions that are less conducive to mite proliferation.

CHAPTER 6

CONCLUSION

This experimental research proved that the flower extract of the *Calendula officinalis* plant, popularly known as pot marigold, does not contain any toxic compounds necessary to kill the *Demodex folliculorum* mite, which has been considered both a friend and an adversary of humans since ancient times. Even at the maximum concentration the *Calendula officinalis* (100 w/v) extract failed to bring acaricidal effect thus proving no role in mite eradication. Thus, the effectiveness of the herbal plants is proved to be inferior to that of ivermectin 1%. The anti-*Demodex* properties of the *Calendula* had a similar effect to that of immersion oil and coconut oil, which have no effect on *Demodex* mite.

It won't be possible to see any eradication of *Demodex* with the application of *Calendula officinalis* on the skin since it doesn't contain Terpinen-4-ol (T4O), an acaricidal intense poisonous chemical found in Thai herbal tea tree oil. A holistic alteration of the skin microbiota, pH of the skin, immunity of the body, gut-skin axis along with *Demodex* proliferation is required for the onset of demodicosis, such as acne rosacea, acne vulgaris and other dermatosis. The study also concluded that only the proliferation of *Demodex* mites is not sufficient to produce *Demodex*-associated dermatitis on the face. Even the presence of few numbers of *Demodex* mite can trigger inflammatory reaction during alteration of above factors.

Considering this, it is important to remember that the management of demodicosis should also consider anti-inflammatory medications. Since the plant has diverse phytochemicals, the floral exact can be mobilized to control the onset of inflammatory cascade observed during the demodicosis. Thus, the plant can be used to control the inflammatory processes like erythema, itching, and pain during *Demodex*-associated facial dermatitis like acne rosacea, making it an additive treatment therapy. *Calendula*, which was long thought to be an antimicrobial agent, has not been found to be effective in the elimination of *Demodex* in demodicosis, although it is potent against certain other microbes like bacteria and helminths.

Other powerful acaricidal agents, such as tea tree oil, ivermectin, metronidazole, retinoid etc, should be prioritized for the purpose of mite eradication. However, because of the anti-inflammatory phytochemicals present in *Calendula officinalis*, the extract can still be utilized to reduce the inflammation on the skin in demodicosis with a view to keep the skin environment in a balanced state and to prevent the excess *Demodex* proliferation. These special properties of the *Calendula officinalis* make it adjunct treatment for the suppression of inflammation and thereby enhancing the parasitostatic effect of *Demodex* mites and still allowing the *Demodex* to live as commensal without posing any threat to human skin.

REFERENCES

Aghazadeh, H., Rigi, A., Sangchooli, T., Taheri, P., Nasiraei, A. H., & Mohammadi, M. (2023). A New Herbal Medicine Formulation with Potential Anti-scabies Properties to Treat Demodex and Sarcoptes Parasites. *Archives of Razi Institute*, *78*(5), 1472-1482.

Ahmed, H. F., Seleiman, M. F., Mohamed, I. A., Taha, R. S., Wasonga, D. O., & Battaglia, M. L. (2023). Activity of essential oils and plant extracts as biofungicides for suppression of soil-borne fungi associated with root rot and wilt of marigold (Calendula officinalis L.). *Horticulturae*, *9*(2), 222.

Al-Snafi, A. E. (2015). The chemical constituents and pharmacological effects of Calendula officinalis-A review. *Indian Journal of Pharmaceutical Science & Research*, *5*(3), 172-185.

Alex, M. M., Criton, V. S., & Mohanan, S. (2023). Papulopustular periorbital demodicosis: A diagnosis often overlooked. *Journal of Skin and Sexually Transmitted Diseases*, *5*(1), 47-49.

Alsoufi, A. S. M., Pączkowski, C., Długosz, M., & Szakiel, A. (2019). Influence of selected abiotic factors on triterpenoid biosynthesis and saponin secretion in marigold (Calendula officinalis L.) in vitro hairy root cultures. *Molecules*, *24*(16), 2907.

Antelo, D. A. P., & da Costa Rocha, L. (2016). Cosmetic Approach in Patients with Acne and Rosacea. *Daily Routine in Cosmetic Dermatology*, 1-28.

Ashwlayan, V. D., Kumar, A., Verma, M., Garg, V. K., & Gupta, S. K. (2018). Therapeutic Potential of Calendula officinalis. *Pharmacy & Pharmacology International Journal*, *6*(2). https://doi.org/10.15406/ppij.2018.06.00171

Bezabh, S. A., Tesfaye, W., Christenson, J. K., Carson, C. F., & Thomas, J. (2022). Antiparasitic activity of tea tree oil (TTO) and its components against medically important ectoparasites: A systematic review. *Pharmaceutics*, *14*(8), 1587.

Bhupathyraaj, M., & Amaresh, N. (2014). Phytochemistry and Pleiotropic Pharmacological Properties of Calendula officinalis - A Review. *Research And Reviews: Journal Of Pharmacognosy And Phytochemistrye*, *2*, 1-10.

Bilušić, T., Šola, I., & Čikeš Čulić, V. (2024). Identification of Flavonoids, Antioxidant and Antiproliferative Activity of Aqueous Infusions of Calendula officinalis L., Chelidonium majus L., Teucrium chamaedrys L. and Alchemilla vulgaris L. *Food technology and biotechnology*, *62*(1), 49-58.

Bøge-Rasmussen, T., Christensen, J., Gluud, B., Kristensen, G., & Norn, M. (1982). Demodex folliculorum hominis (Simon): incidence in a normomaterial and in patients under systemic treatment with erythromycin or glucocorticoid. *Acta Dermato-Venereologica*, *62*(5), 454-456.

Chandy, A. (2023). Comprehensive Overview of Medicinal Plants Utilized for Treatment of Skin Disorders. *Pharmaceutical and Biosciences Journal*, 01-11.

Chudzicka-Strugała, I., Gołębiewska, I., Brudecki, G., Elamin, W., & Zwoździak, B. (2023). Demodicosis in different age groups and alternative treatment options—A review. *Journal of Clinical Medicine*, *12*(4), 1649.

Della Loggia, R., Tubaro, A., Sosa, S., Becker, H., & Isaac, O. (1994). The role of triterpenoids in the topical anti-inflammatory activity of Calendula officinalis flowers. *Planta medica*, *60*(06), 516-520.

Demirci, G. (2023). *Comparative Demonstration of the Acaricidal Effects of Heat and Ivermectin% 1 on Demodex Folliculorum using in Vitro Camera Microscopy.* ResearchGate.

Devkota, H. P. (2022). An overview of medicinal plants of the Asteraceae family and their role in human health. *Medicinal plants of the asteraceae family: traditional uses, phytochemistry and pharmacological activities*, 1-15.

Dey, H., Tarannum, M., Kumar, P., Agarwal, V., Parvez, E., Ali, M. N., Shafieq, S., & Sharma, V. (2024). Calendula officinalis-A Crucial and Promising Antifungal Agent with Cytotoxic Biological Attributes. *Sch Acad J Pharm*, *1*, 30-36.

Dhingra, G., Dhakad, P., & Tanwar, S. (2022). Review on phytochemical constituents and pharmacological activities of plant Calendula officinalis Linn. *Biological Sciences*, *2*(2), 216-228.

Dopytalska, K., Lipa, K., Sobolewski, P., Szymańska, E., & Walecka, I. (2019). Role of Demodex folliculorum in dermatology. *Dermatology Review/Przegląd Dermatologiczny*, *106*(5), 507-514.

Efstratiou, E., Hussain, A. I., Nigam, P. S., Moore, J. E., Ayub, M. A., & Rao, J. R. (2012). Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. *Complementary Therapies in Clinical Practice*, *18*(3), 173-176.

Elston, C. A., & Elston, D. M. (2014). Demodex mites. *Clin Dermatol*, *32*(6), 739-743. https://doi.org/10.1016/j.clindermatol.2014.02.012

Ferhatosmanoğlu, A., Selçuk, L. B., Arıca, D. A., & Kapan, O. (2023). The relationship of Demodex density with acne severity. *Turkish Journal of Dermatology*, *17*(2), 52-56.

Foley, R., Kelly, P., Gatault, S., & Powell, F. (2021). Demodex: a skin resident in man and his best friend. *Journal of the European Academy of Dermatology and Venereology*, *35*(1), 62-72.

Forton, F. M., & De Maertelaer, V. (2021). Which factors influence Demodex proliferation? A retrospective pilot study highlighting a possible role of subtle immune variations and sebaceous gland status. *The Journal of Dermatology*, *48*(8), 1210-1220.

Garrido-Suárez, B. B., Garrido, G., Menéndez, A. B., Merino, N., Valdés, O., de la Paz, N., . . . Piñeros, O. (2023). Topical Calendula officinalis L. inhibits inflammatory pain through antioxidant, anti-inflammatory and peripheral opioid mechanisms. *Journal of Integrative Medicine*, *21*(1), 34-46.

Ge, Q., Zhao, S., Shao, X., Wei, Y., Chen, J., Wang, H., & Xu, F. (2024). Influence of flavonoids from Sedum aizoon L. on mitochondrial function of Rhizopus nigricans in strawberry. *World Journal of Microbiology and Biotechnology*, *40*(5), 161.

Godara, R., Katoch, R., Yadav, A., Ahanger, R., Bhutyal, A., Verma, P., Katoch, M., Dutta, S., Nisa, F., & Singh, N. (2015). In vitro acaricidal activity of ethanolic and aqueous floral extracts of Calendula officinalis against synthetic pyrethroid resistant Rhipicephalus (Boophilus) microplus. *Experimental and Applied Acarology*, *67*, 147-157.

Goncalves, A. C., Castro, S., Paiva, J., Santos, C., & Silveira, P. (2018). *Taxonomic revision of the genus Calendula (Asteraceae) in the Iberian Peninsula and the Balearic Islands*. CABI.

Gothe, R. (1989). Demodicosis of dogs--a factorial disease? *Berliner und Munchener tierarztliche Wochenschrift*, *102*(9), 293-297.

Hachmi, A., Zbiri, A., & El Alaoui-Faris, F. E. (2023). Investigation of Flora, Soil, and Biodiversity of Ecosystems in Arid Eastern Moroccan Rangelands. *Ekológia (Bratislava)*, *42*(2), 122-132.

Heczko, J., Schell, C., Pansick, A., Stein, R., & Perry, H. D. (2023). Evaluation of a novel treatment, selenium disulfide, in killing Demodex folliculorum in vitro. *Canadian Journal of Ophthalmology*, *58*(5), 408-412. https://doi.org/https://doi.org/10.1016/j.jcjo.2022.04.012

Hernández-Díaz, J. A., Garza-García, J. J., León-Morales, J. M., Zamudio-Ojeda, A., Arratia-Quijada, J., Velázquez-Juárez, G., . . . García-Morales, S. (2021). Antibacterial activity of biosynthesized selenium nanoparticles using extracts of Calendula officinalis against potentially clinical bacterial strains. *Molecules*, *26*(19), 5929.

Hooda, P., Malik, R., Bhatia, S., Al-Harrasi, A., Najmi, A., Zoghebi, K., . . . Mohan, S. (2024). Phytoimmunomodulators: A review of natural modulators for complex immune system. *Heliyon*, *10*(1).

Hu, L., Guan, C., Zhao, Y., Zhang, W., Chai, R., Teng, J., . . . Wu, F. (2023). Cloning, sequencing, expression, and purification of aspartic proteases isolated from two human Demodex species. *International Journal of Biological Macromolecules*, *253*, 127404.

Izdebska, J. N., Rolbiecki, L., & Fryderyk, S. (2016). A new species of Demodex (Acari: Demodecidae) from the skin of the vibrissal area of the house mouse Mus musculus (Rodentia: Muridae), with data on parasitism. *Systematic and Applied Acarology*, *21*(8), 1031-1039.

Jodh, R., Tawar, M., Behere, S., Randhave, N., Jirapure, P., & Ingle, S. (2023). A Review on Calendula officinalis. *Research Journal of Pharmacognosy and Phytochemistry*, *15*(1), 5-10.

Kairey, L., Agnew, T., Bowles, E. J., Barkla, B. J., Wardle, J., & Lauche, R. (2023). Efficacy and safety of Melaleuca alternifolia (tea tree) oil for human health—A systematic review of randomized controlled trials. *Frontiers in pharmacology*, *14*, 1116077.

Kalvatchev, Z., Walder, R., & Garzaro, D. (1997). Anti-HIV activity of extracts from Calendula officinalis flowers. *Biomedicine & pharmacotherapy*, *51*(4), 176-180.

Kargadouri, A., Beleri, S., & Patsoula, E. (2024). Data on Demodex Ectoparasite Infestation in Patients Attending an Outpatient Clinic in Greece. *Parasitologia*, *4*(2), 129-136.

Kiaei, N., Hajimohammadi, R., & Hosseini, M. (2018). Investigation of the anti-inflammatory properties of Calendula nanoemulsion on skin cells. *Bioinspired, Biomimetic and Nanobiomaterials*, *7*(4), 228-237.

Lam, N. S. K., Long, X. X., Griffin, R. C., Chen, M.-K., & Doery, J. C. (2018). Can the tea tree oil (Australian native plant: Melaleuca alternifolia Cheel) be an alternative treatment for human demodicosis on skin? *Parasitology*, *145*(12), 1510-1520.

Lam, N. S. K., Long, X. X., Li, X., Yang, L., Griffin, R. C., & Doery, J. C. (2020). Comparison of the efficacy of tea tree (Melaleuca alternifolia) oil with other current pharmacological management in human demodicosis: A Systematic Review. *Parasitology*, *147*(14), 1587-1613.

Li, J., Wei, E., Reisinger, A., French, L. E., Clanner-Engelshofen, B. M., & Reinholz, M. (2023). Comparison of different anti-Demodex strategies: a systematic review and meta-analysis. *Dermatology*, *239*(1), 12-31.

Li, Y. M., Milikowski, C., & Galimberti, F. (2021). Case of bullous Grover disease. *The American journal of dermatopathology*, *43*(2), 141-143.

Lohani, A., Verma, A., Hema, G., & Pathak, K. (2021). Topical Delivery of Geranium/Calendula Essential Oil-Entrapped Ethanolic Lipid Vesicular Cream to Combat Skin Aging. *BioMed Research International*, *2021*(1), 4593759.

Mastrota, K. (2023). Demodex “tails”. *Optometry Times Journal, 15*(7).

Millikan, L. E. (2001). *Androgenetic alopecia: the role of inflammation and Demodex.* CABI.

Nasir, N. A. M. M., Abllah, Z., Jalaludin, A. A., Shahdan, I. A., & Abd Manan, W. N. H. W. (2018). Virgin coconut oil and its antimicrobial properties against pathogenic microorganisms: a review. International dental conference of sumatera utara 2017 (IDCSU 2017),

Ogden, E. E. (n.d.). *All About Calendula*. Retrieved September 9, 2022, from https://www.americanmeadows.com/content/wildflower-seeds/wildflower-species/all-about-calendula

Ozdemir Cetinkaya, P., Özkesici Kurt, B., Altunay, I. K., & Aksu Cerman, A. (2024). Which factors influence Demodex mite density in standardized superficial skin biopsy in patients with rosacea? A prospective cross-sectional analysis. *Archives of Dermatological Research*, *316*(6), 1-10.

Paichitrojjana, A. (2022). Demodex : The worst enemies are the ones that used to be friends. *Dermatology reports*, *14*(3), 9339. https://doi.org/10.4081/dr.2022.9339

Paichitrojjana, A., & Chalermchai, T. (2023). Comparison of in vitro Killing Effect of Thai Herbal Essential Oils, Tea Tree Oil, and Metronidazole 0.75% versus Ivermectin 1% on Demodex folliculorum. *Clin Cosmet Investig Dermatol*, *16*, 1279-1286. https://doi.org/10.2147/CCID.S414737

Paichitrojjana, A., & Chalermchai, T. (2024). The Association Between Acne Vulgaris, Acne Vulgaris with Nonspecific Facial Dermatitis, and Demodex Mite Presence. *Clinical, Cosmetic and Investigational Dermatology*, 137-146.

Paichitrojjana, A., & Paichitrojjana, A. (2022). Successful treatment of ivermectin refractory demodicosis with isotretinoin and permethrin cream. *JAAD Case Reports*, *26*, 98-100.

Paichitrojjana, A., & Paichitrojjana, A. (2023). Case Series of Demodicosis in Acne Vulgaris Patients. *Clin Cosmet Investig Dermatol*, *16*, 3363-3368. https://doi.org/10.2147/ccid.S441581

Parać, E., Špiljak, B., Lugović-Mihić, L., & Bukvić Mokos, Z. (2023). Acne-like Eruptions: Disease Features and Differential Diagnosis. *Cosmetics*, *10*(3), 89.

Patil, K., Sanjay, C., DoggALLI, N., Devi, K., & Harshitha, N. (2022). A Review of Calendula OfficinalisMagic in Science. *Journal of Clinical and Diagnostic Research*, *16*(2), ZE23-ZE27.

Peron, G., Franceschi, C., Da Dalt, C., Ferrarese, I., Sut, S., & Dall'Acqua, S. (2024). Biostimulation of Calendula officinalis with a soy protein hydrolysate induces flower and plant biomass and flower count by reversibly altering the floral metabolome. *Industrial Crops and Products*, *214*, 118508.

Prasher, P., Baghra, D., Singh, D., Thakur, S., Gill, N. K., & Kesavan, A. K. (2020). Molecular identification and phylogenetic relationship of Demodex mites based on mitochondrial 16S rDNA. *Trop Parasitol*, *10*(2), 136-141. https://doi.org/10.4103/tp.TP_76_19

Rather, P. A., & Hassan, I. (2014). Human demodex mite: the versatile mite of dermatological importance. *Indian J Dermatol*, *59*(1), 60-66. https://doi.org/10.4103/0019-5154.123498

Ravichandran, S., Bhargavi, K. M., Rai, A., Pandey, T., Rajput, J., & Sri, R. M. (2023). Medicinal plants for curing human diseases. *Insight-Chinese Medicine*, *6*(1), 570-570.

Rezai, S., Rahzani, K., Hekmatpou, D., & Rostami, A. (2023). Effect of oral Calendula officinalis on second-degree burn wound healing. *Scars, Burns & Healing*, *9*, 20595131221134053.

Rigane, G., Younes, S. B., Ghazghazi, H., & Salem, R. B. (2013). Investigation into the biological activities and chemical composition of Calendula officinalis L. growing in Tunisia. *International Food Research Journal*, *20*(6), 3001.

Samatadze, T. E., Yurkevich, O. Y., Khazieva, F. M., Basalaeva, I. V., Savchenko, O. M., Zoshchuk, S. A., . . . Muravenko, O. V. (2023). Genome Studies in Four Species of Calendula L.(Asteraceae) Using Satellite DNAs as Chromosome Markers. *Plants*, *12*(23), 4056.

Sarac, G. (2019). A comparison of the efficacy and tolerability of topical agents used in facial Demodex treatment. *Journal of Cosmetic Dermatology*, *18*(6), 1784-1787.

Savla, K., Le, J. T., & Pucker, A. D. (2020). Tea tree oil for Demodex blepharitis. *Cochrane Database of Systematic Reviews*, *6*.

Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Ferreira, M. K. M., . . . Cruz, J. (2023). An updated review on the multifaceted therapeutic potential of Calendula officinalis L. *Pharmaceuticals*, *16*(4), 611.

Silva, D., Ferreira, M. S., Sousa-Lobo, J. M., Cruz, M. T., & Almeida, I. F. (2021). Anti-inflammatory activity of Calendula officinalis L. flower extract. *Cosmetics*, *8*(2), 31.

Sławińska, M., Jaworska, K., Wyszomirski, A., Rychlik, K., Nowicki, R. J., & Sobjanek, M. (2023). Videodermoscopy in the assessment of patients with ocular demodicosis. *Dermatology Practical & Conceptual*, *13*(2).

Suja, S., Aswathy, V., Bijukumar, B., & Prakashkumar, R. (2024). Flavonoids of asteraceae promising agents. *Flavonoids as Nutraceuticals, 121.*

Thao, N. T., Van Ba, H., & Trang, N. T. T. (2023). Research on the prevalence, clinical characteristics, and related factors in acne vulgaris patients with demodex infection in can tho city from 2021 to 2023. *Tạp chí Y Dược học Cần Thơ, 6,* 93-99.

Tong, C. M., & Kurji, K. (2021). Blepharitis: Overview and Classification. *Cornea, E-Book*, *5*, 308.

Trave, I., Cozzani, E., Salvi, I., & Parodi, A. (2023). Follicular scales, scalp and ocular involvement in patients with papulopustular rosacea: prevalence and association with Demodex mite proliferation. *Dermatology Reports*, *16*(1), 9798.

Ullah, M. A., Hassan, A., & Hamza, A. (2023). Calendula (Calendula officinalis) marigold as medicinal plant. *Orthopaedics Case Reports*, *2*(1).

Venkatesh, D. P., Gheena, S., Ramani, P., Rajeshkumar, S., & Ramalingam, K. (2023). In Vitro Evaluation of Antioxidant and Anti-inflammatory Potentials of Herbal Formulation Containing Marigold Flower (Calendula officinalis L.) Tea. *Cureus*, *15*(8).

Wei, F., Li, L., Kong, Y., Yan, X., Varghese, K. J., Zhang, S., . . . Chen, H. (2024). Evidence for the clinical association between Demodex and rosacea: a review. *Dermatology*, *240*(1), 95-102.

Zhang, A. C., Muntz, A., Wang, M. T., Craig, J. P., & Downie, L. E. (2020). Ocular Demodex: a systematic review of the clinical literature. *Ophthalmic and Physiological Optics*, *40*(4), 389-432.

Zhang, J., Saeed, A., Hussain, M., Siddique, R., Xu, H., Bazmi, R. R., . . . Lv, G. (2024). Aqueous Ethanolic Extract of Inonotus obliquus Ameliorates Polycystic Ovarian Syndrome by Modulating Oxidative Stress and Downregulating TNF-α and Interleukin-6. *Journal of Food Biochemistry*, *2024*(1), 1003797.

Zournatzis, I., Liakos, V., Papadopoulos, S., & Wogiatzi, E. (2025). Calendula officinalis - A comprehensive review. *Pharmacological Research - Natural Products*, *6*, 100140. https://doi.org/https://doi.org/10.1016/j.prenap.2024.100140

APPENDIX A

ETHIC

Memorandum

Office Research Administration Division, Mae Fah Luang University Research and Innovation Institute **Phone No.** 7171 (Sirintip)

MFU 7742(1)/2782 **Date** 7 August 2024

Subject Notification for the result of protocol and related documents

To Mr.Nabin Rokaya

According to the principal investigator, Mr. Nabin Rokaya, a student from the School of Anti-Aging and Regenerative Medicine sent a research project entitled "The efficacy of calendula officinalis extract on the mortality of Demodex folliculorum", protocol code: **EC 24149-20.**

The Mae Fah Luang University Ethics Committee on Human Research (MFU EC) has received all documents since August 5, 2024, and the consideration result is

Exemption

Please contact to receive a certificate of exemption (COE) at the office of MFU EC, 4th floor, Academic Services Building (AS), by making an appointment in advance with Miss Sirintip Arintasai at telephone number 0-5391-7171 or 0-5391-7170

For your consideration and further implementation.

Best Regards,

(Asst. Prof. Dr. Sivaporn Sivasinprasasn)

Committee and Secretary

The Mae Fah Luang University Ethics Committee on Human Research

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

หนังสือยกเว้นการพิจารณาด้านจริยธรรมการวิจัย

COE: 174/2024 **รหัสโครงการวิจัย: EC 24149-20**

ชื่อโครงการวิจัย: งานวิจัยเกี่ยวกับประสิทธิผลของสารสกัด Calendula officinalis ต่ออัตราการตายของ Demodex folliculorum

ชื่อผู้วิจัยหลัก: นายนาบิน โรคะยา

สำนักวิชา: เวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง พิจารณาโครงร่างการวิจัย โดยยึด แนวทางจริยธรรมสากล ได้แก่ ปฏิญญาเฮลซิงกิ (Declaration of Helsinki) รายงานเบลมองต์ (Belmont Report) แนวทางจริยธรรมสากลสำหรับการวิจัยในมนุษย์ขององค์การสากลด้านวิทยาศาสตร์การแพทย์ (CIOMS) และแนวทางการปฏิบัติการวิจัยที่ดี (ICH GCP) ได้พิจารณาแล้วเห็นว่า โครงการวิจัยดังกล่าวข้างต้น เข้าข่ายยกเว้นการพิจารณาด้านจริยธรรมการวิจัย

วันที่รับรองยกเว้นการพิจารณาด้านจริยธรรมการวิจัย: 5 กันยายน 2567

ลงนาม .....

(อาจารย์ นพ.จุลพงศ์ อจลพงศ์)

ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง

ผู้วิจัยที่โครงร่างการวิจัยได้รับยกเว้นการพิจารณาด้านจริยธรรมการวิจัย จากคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง ต้องปฏิบัติดังต่อไปนี้

- ไม่ต้องส่งรายงานความก้าวหน้าของการวิจัย
- ในกรณีที่มีการเปลี่ยนแปลงโครงการวิจัย ส่งแบบรายงานการแก้ไขเพิ่มเติมโครงร่างการวิจัย (AP 06/2022) และโครงร่างการวิจัยที่มีการแก้ไขเพิ่มเติม เพื่อแจ้งให้คณะกรรมการฯ พิจารณาก่อนดำเนินการวิจัยตามที่ต้องการเปลี่ยนแปลง
- ส่งแบบรายงานสรุปผลการวิจัย (AP 09_2022)

หมายเหตุ สามารถ Download แบบรายงานต่าง ๆ ได้ที่ https://ec.mfu.ac.th

ข้าพเจ้าในฐานะ ผู้วิจัย ยินยอมที่จะปฏิบัติตามข้อกำหนดดังกล่าว

.....

(นายนาบิน โรคะยา)

วันที่ 25-10-2024

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

CERTIFICATE OF EXEMPTION

COE: 174/2024 **Protocol No:** EC 24149-20

Title: THE EFFICACY OF CALENDULA OFFICINALIS EXTRACT ON THE MORTALITY OF DEMODEX FOLLICULORUM

Principal investigator: Mr. Nabin Rokaya

School: Anti-Ageing and Regenerative Medicine

The Mae Fah Luang University Ethics Committee on Human Research (MFU EC) reviewed the protocol in compliance with international guidelines such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice (ICH GCP) and decided to exempt the above research protocol.

Date of Exemption: September 5, 2024

.....

(Lecturer Jullapong Achalapong, M.D.)
Vice Chairperson of the MFU Ethics Committee on Human Research

For research protocol exempted by the Mae Fah Luang University Ethics Committee on Human Research (MFU EC), the investigators must comply with the followings:

- No need to submit a progress report.
- When there are changes of the protocol, the investigator must send an amendment report (AP 06/2022) to the MFU EC.
- When the research finishes, the investigator must send a final report (AP 09/2022).

Please go to https://ec.mfu.ac.th to download MFU EC forms for reporting.

I, as an investigator, agree to comply with the above obligation.

.....

Mr. Nabin Rokaya
Date 25-10-2024

APPENDIX B

INFORMED CONSENT FORM

หนังสือแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัย สำหรับอาสาสมัคร

ชื่อโครงการวิจัย–การศึกษาประสิทธิผลของสารสกัดดาวเรืองในการทำลายเชื้อเดโมเด็กติกฟอลลิคุโลรุม

THE EFFICACY OF CALENDULA OFFICINALIS EXTRACT ON THE MORTALITY OF DEMODEX FOLLICULORUM

ข้าพเจ้า นาย/นาง/นางสาว..... ที่อยู่..... ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัยวิจัย ฉบับวันที่.....

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย และสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

..... ลงนามผู้เข้าร่วมในโครงการวิจัย

(.....) ชื่อ-สกุล ผู้เข้าร่วมในโครงการวิจัย (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้นประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

..... ลงนามผู้วิจัย

(Nabin Rokaya) ชื่อ-สกุล ผู้วิจัย (ตัวบรรจง)

..... ลงนามพยาน

(.....) ชื่อ-สกุล พยาน (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

APPENDIX C

RESEARCH FORM

Comparative study of Different Agents on the Viability of *Demodex* mite

Form 1:

Name of Testing Agents (Topical formulation)	**Mortality of *Demodex* at different periods of time in vitro**							
	Viability lost within							Inference
	Baseline	1 hr	2 hr	3 hr	4 hr	5 hrs	6 hrs	
Ivermectin 1% (Positive Control)								
Immersion Oil (Negative Control)								
Chemical Gel								
Calendula extract 5%								
Calendula extract 2%								
Calendula extract 10%								
Cal. (other conc)								

Form 2:

Viability testing at different point of time

Time Period	***Calendula* extract**				**Ivermectin** (1%) (Positive control)	**Immersion oil** (Negative Control)	Gel	**Inference** (Live/dead)
Baseline	2%	5%	10%	others				
	Introduction of Different Testing Agents **No. of viable *Demodex* Count (Demodex pop)**							
30 mins								
1 hr								
1.5 hr								
2 hr								
2.5 hr								
3 hr								
4 hr								
5 hr								
6 hr								

- ☐ Total No of Live mites at the end of the experiment: …
- ☐ Total No of Dead Mites at the end of the experiment: …
- ☐ Total No of Discarded mites during the experiment…

Form 3:

3-cycle of repeated testing of the parasiticidal effect of *Calendula* extract

		Different concentration Of *Calendula* extract			
		Calendula extract	*Calendula* extract	*Calendula* extract	Inference
	Concentration amount	2% (w/v)	5%	10%	
	After the introduction of Different testing Drugs No. of viable *Demodex* Count (*Demodex* population)				
Time period	Baseline (0 min)				
	30 mins				
	1 hour				
	1.5 hour				
	2 hours				
	2.5 hour				
	3 hours				
	4 hours				
	5 hours				
	6 hours				

- ☐ Total No of Live mites at the end of the experiment: …
- ☐ Total No of Dead Mites at the end of the experiment: …
- ☐ Total No of Discarded mites during the experiment…

Form 4:

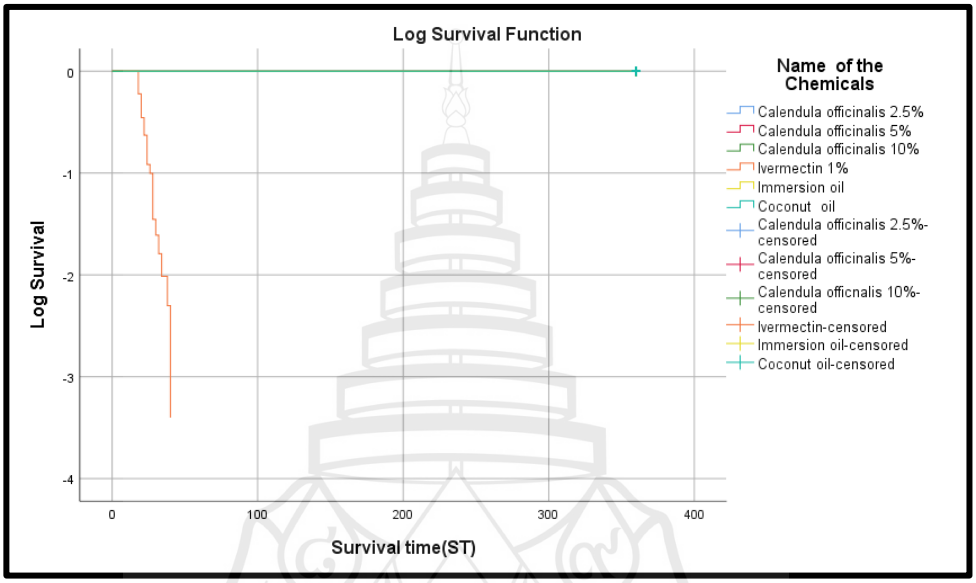
Experiment Time period record form (each experiment)

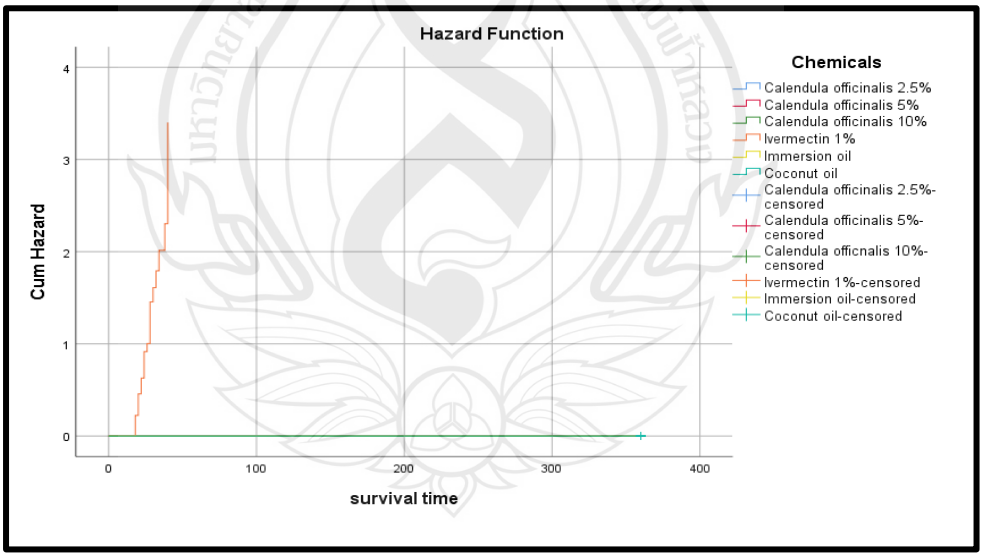
Time of the day (Clock)							
Experiment Hour	0 min	30 mins	1 hour	2 hours	3 hours	4 hours	>4 hour
Total hour	←						→

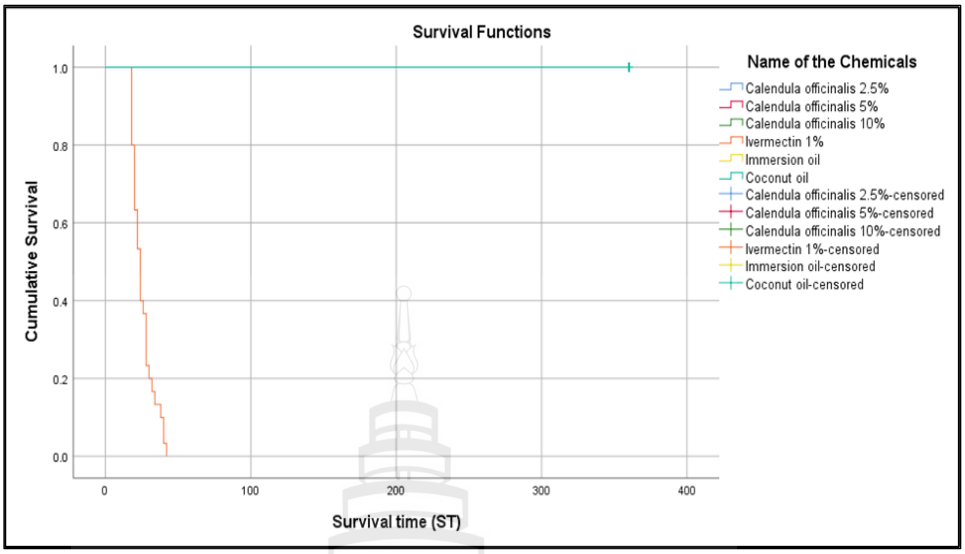
Room Temperature: ……

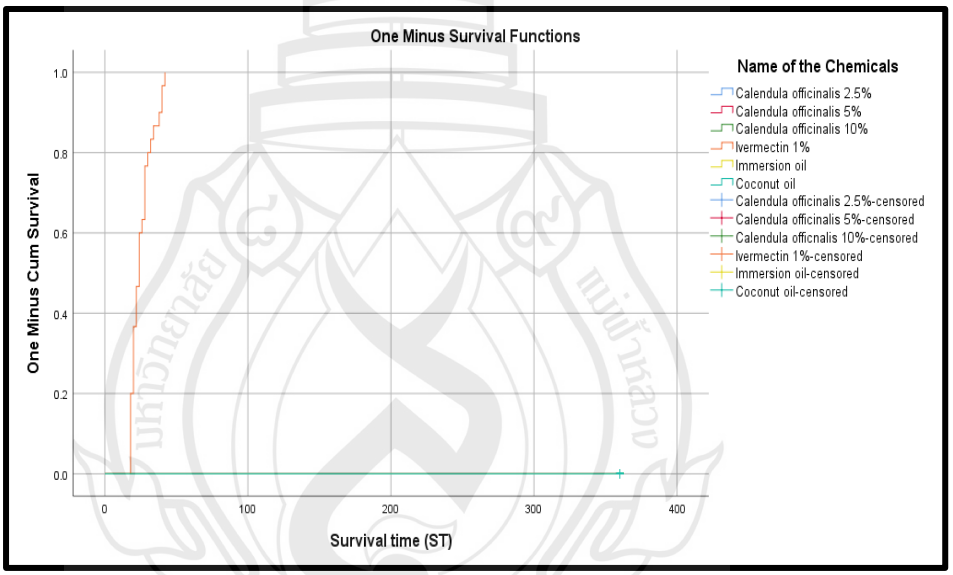
APPENDIX D

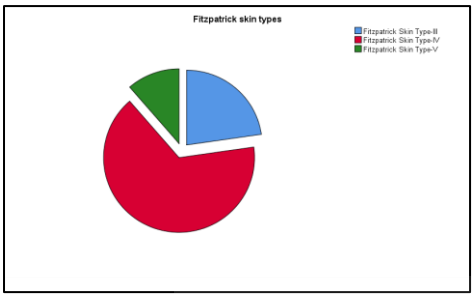
FIGURES

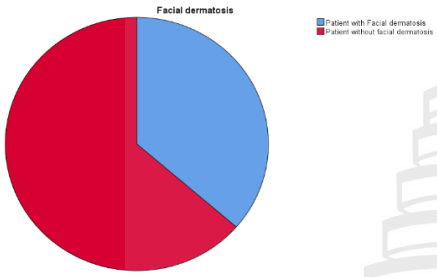


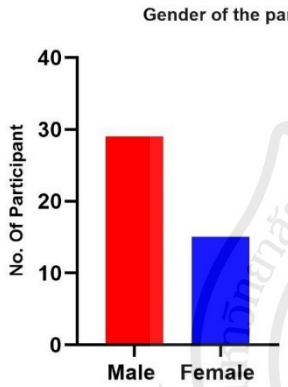


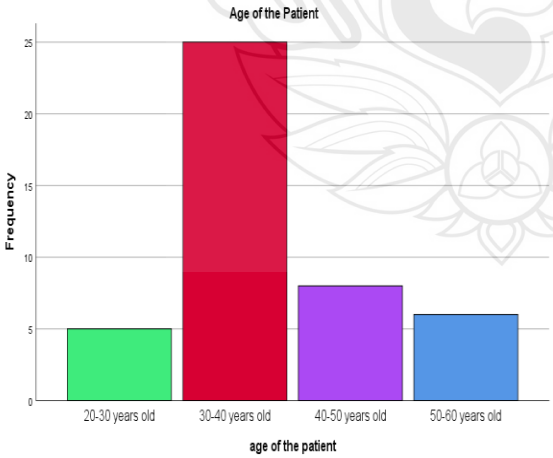

Survival Functions
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
Survival time (ST)
Name of the Chemicals
Calendula officinalis 2.5%
Calendula officinalis 5%
Calendula officinalis 10%
Ivermectin 1%
Immersion oil
Coconut oil
Calendula officinalis 2.5%-censored
Calendula officinalis 5%-censored
Calendula officinalis 10%-censored
Ivermectin 1%-censored
Immersion oil-censored
Coconut oil-censored


One Minus Survival Functions
One Minus Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
Survival time (ST)
Name of the Chemicals
Calendula officinalis 2.5%
Calendula officinalis 5%
Calendula officinalis 10%
Ivermectin 1%
Immersion oil
Coconut oil
Calendula officinalis 2.5%-censored
Calendula officinalis 5%-censored
Calendula officinalis 10%-censored
Ivermectin 1%-censored
Immersion oil-censored
Coconut oil-censored


Fitzpatrick skin types
Fitzpatrick Skin Type-III
Fitzpatrick Skin Type-IV
Fitzpatrick Skin Type-V


Facial dermatosis
Patient with Facial dermatosis
Patient without facial dermatosis


Gender of the participant
No. Of Participant
40
30
20
10
0
Male
Female


Age of the Patient
Frequency
25
20
15
10
5
0
20-30 years old
30-40 years old
40-50 years old
50-60 years old
age of the patient

APPENDIX E

MATERIALS AND REAGENT FOR THE EXPERIMENT

Figure E1 Testing chemicals

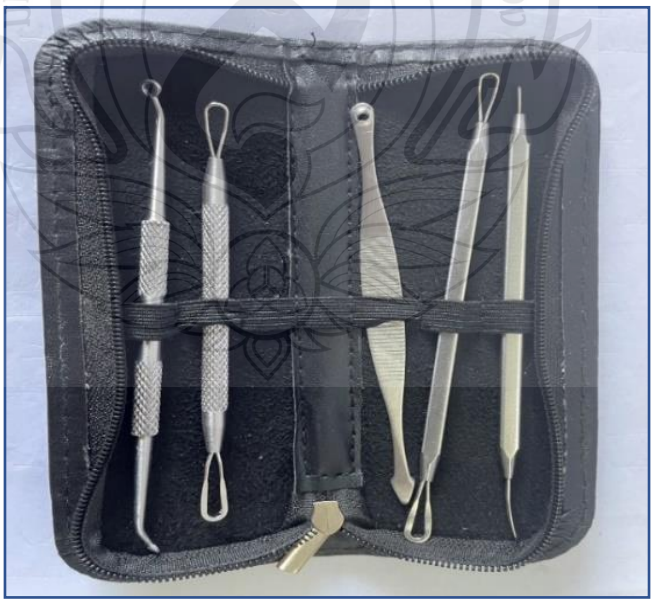


Figure E2 Acne extractor

Figure E3 Ivermectin 1% solution

Figure E4 Glass slides

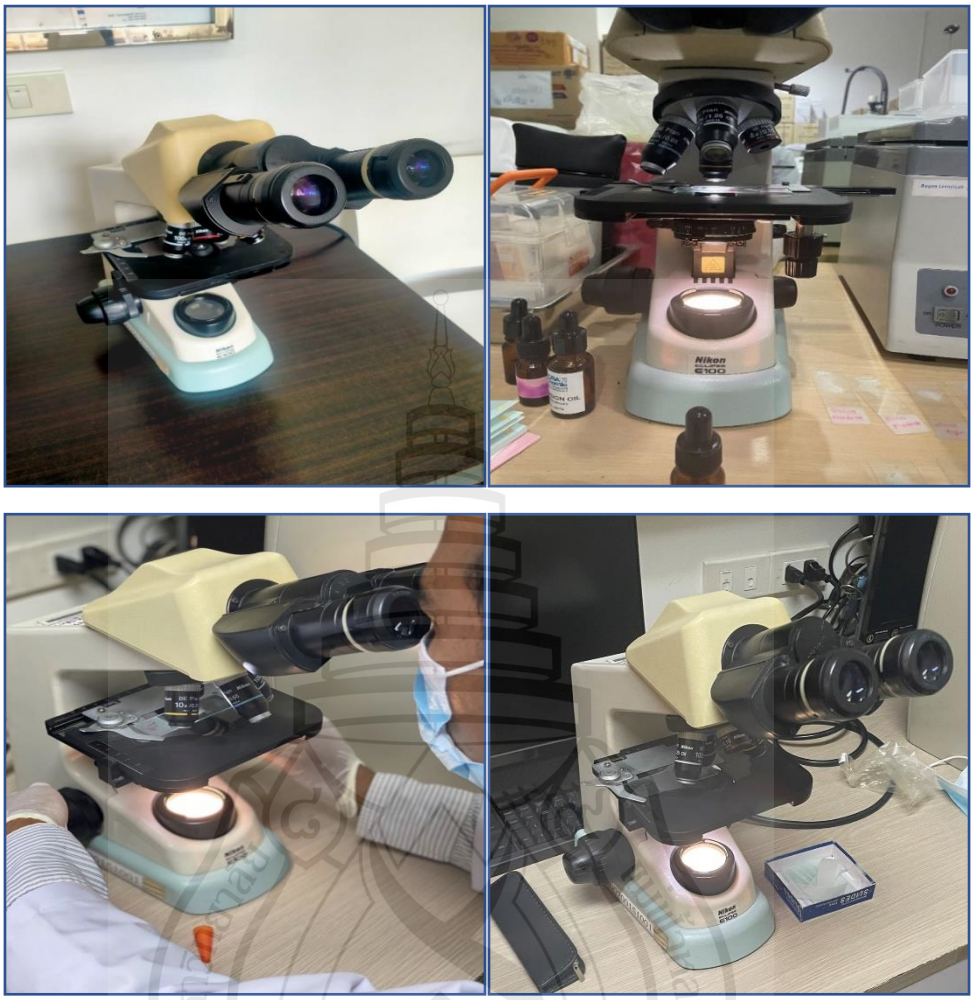


Figure E5 Microscope (Nikon Eclipse E100, Japan)

Figure E6 SSSB method of *Demodex folliculorum* mites extraction

Figure E6 (continued)

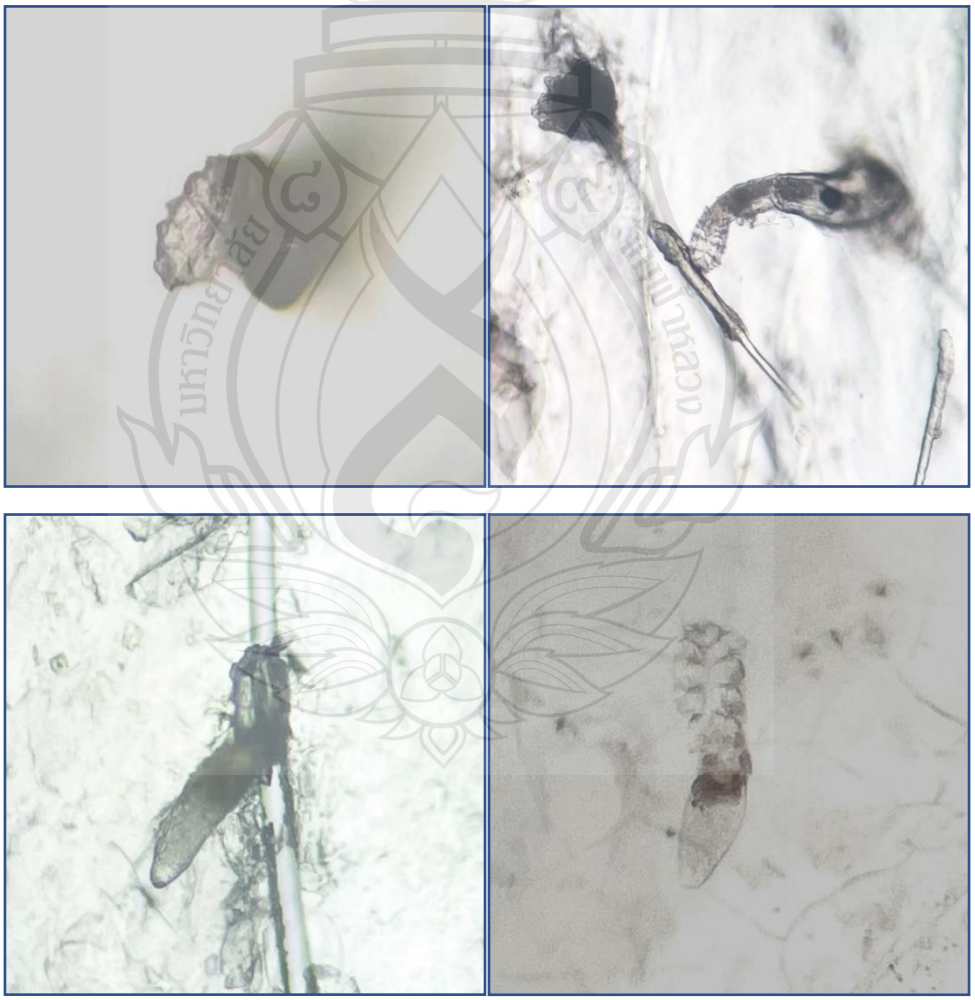


Figure E7 *Demodex folliculorum* mites observed under Microscope

CURRICULUM VITAE

NAME Nabin Rokaya

EDUCATIONAL BACKGROUND

2024 United States Medical Licensing Exam:USMLE Step-1

2017 Bachelor of Medicine and Bachelor of Surgery (MBBS), Tribhuvan University, Nepal

WORK EXPERIENCE

2016-2017 Internship
Universal College of Medical Sciences, Bhairahawa, Nepal

2017-2022 Medical Officer
District Hospital, Simikot, Humla, Nepal

PUBLICATIONS

Alam, M. R., Rokaya, N., Mahat, S., Upadhyaya, A., & Rokaya, P. (2022). A Rare Presentation of Hand, Foot, and Mouth Disease During Pregnancy. *Cureus, 14*(8).

Karki, M., Rokaya, N., Khatri, P., Shahi, A., & Sah, A. (2019). Study of Serum Uric Acid Level in Acute Coronary Syndrome in Nepal Subjects. *Trends Journal of Sciences Research, 4*(1), 21-28.

Mahat, S., Manandhar, U., Thapa, P., Rokaya, N., & Alam, M. R. (2023). Case Report: Neglected case of duodenal strongyloidiasis in an immunocompromised patient. *F1000Research, 12,* 307.

Rokaya, D., Rokaya, N., Patel, J. N., Sah, A., Yadav, R. K., & Suttagul, K. (2022). Health Problems and Challenges for Optimal Health-Care Delivery in Humla District, Nepal. *Journal of Datta Meghe Institute of Medical Sciences University, 17*(3), 616-619.

Rokaya, D., Suttagul, K., Karki, S., Rokaya, N., Seriwatanachai, D., & Humagain, M. (2017). A survey on oral health and practice of Nepalese in areas affected by earthquake in 2015. *Kathmandu Univ Med J, 15*(57), 45-50.

Shrestha, U., Rokaya, N., Rokaya, D., Suttagul, K., Shah, P. K., Humagain, M., . . . Seriwatanachai, D. (2018). Post'Gorkha earthquake'medical problems of the victims in nepal. *Journal of International Dental and Medical Research, 11*(1), 71-75.

Skallevold, H. E., Rokaya, N., Wongsirichat, N., & Rokaya, D. (2023). Importance of oral health in mental health disorders: An updated review. *Journal of Oral Biology and Craniofacial Research, 13*(5), 544-552.

Suttagul, K., Rokaya, D., Rokaya, N., & Humagain, M. (2016). Prevalence of dental caries and oral hygiene practice in school children of Bhaktapur, Nepal. *World Journal of Medical Sciences, 13*(2), 107-10.