

Formulation and Evaluation of Antifungal Herbal Soaps using Natural Ingredients by Melt and Pour Method

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

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Declaration

We hereby declare that the work presented in the thesis entitled "Formulation and Evaluation of Antifungal Herbal Soaps using Natural Ingredients by Melt and Pour Method", submitted by us to the Sathyabama Institute of Science & Technology, Chennai-600119 for the award of the degree of Bachelor of Pharmacy (2019-2023) by Research is an original record of research work carried out by us under the supervision of "Dr.Joan Vijetha R, Associate professor, School of Pharmacy". The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associate-ship etc.. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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ABSTRACT

People are most frequently affected by microbial skin infections, which necessitate intensive care both for treatment and to preserve healthy skin. The purpose of the current study is to develop antifungal formulations using some herbal plant extracts that show antifungal activity. The extraction was performed using cold maceration method. Since, most commercial soaps which are available in market contain chemicals that can be harmful to the skin and usage of a natural herbal soap can be a good alternative. The herbal soap was formulated by using *Macroptilium Lathyroides* and *Acalyph Indica* by using melt and pour method. The prepared formulations were evaluated for various physicochemical parameters and were found satisfactory. Also, the evaluation tests showed that the herbal soap has satisfactory antifungal results. And the comparison analysis revealed that the proposed formulation had good results.

Keywords: Antifungal, *Macroptilium Lathyroides*, *Acalypha Indica*, Herbal soap, antifungal, *Candida albicans* & *Non albican Candida sps.*

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LIST OF ABBREVIATIONS

0C	Degree Celsius
mL	Milliliter
mm	Millimeter
μL	Microliter
UV	Ultraviolet
spp	<i>Species</i>
cm	Centimeter
hrs	Hours
HIV	Human Immunodeficiency Virus
B.C.	Before Christ
ZnO	Zinc Oxide
UV-Vis	Ultraviolet Visible
XRD	X-Ray Diffraction
FT-IR	Fourier Transform Infrared
SEM	Scanning Electron Microscopy
EDAX	Energy Dispersive X-Ray Analysis
DLS	Division of Laboratory System
Tio2	Titanium Dioxide

W/W	Weight by Weight
FESEM	Field Emission Scanning Electron Microscopy
SDB	Sabouraud Dextrose Broth
SDA	Sabouraud Dextrose Agar
CC	Carrier Solvent Control
PC	Positive Control
ZOI	Zone Of Inhibitor
PMNL	Polymorphonuclear Leukocytes

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CHAPTER- 1

INTRODUCTION

Fungal skin infections are a type of infection caused by fungi that grow on or in the skin. Fungal infections can occur on any part of the body, but they are most commonly found on the feet, nails, groin, and scalp¹. These infections are also known as mycoses or dermatophytosis, and they are caused by several types of fungi, including dermatophytes, yeasts, and molds. Fungal infections on the skin can cause a range of symptoms, including itching, redness, scaling, cracking, and blisters². In severe cases, the infection can lead to pain and discomfort, and it can even cause permanent damage to the skin and nails. Some types of fungal infections are also contagious, meaning they can spread from person to person through direct contact or through contaminated objects like towels or clothing. There are several factors that can increase the risk of developing a skin fungal infection, including poor hygiene, a weakened immune system, exposure to moist or humid environments, and wearing tight or restrictive clothing. Treatment for fungal infections usually involves antifungal medications, either topical or oral, and can vary depending on the type and severity of the infection. Prevention measures include maintaining good hygiene practices, avoiding sharing personal items, and keeping the skin dry and cool³.

Fungal infection of the skin is very common in India. In the Department of Dermatology, Stanley Medical College and hospital where the study was conducted on Patients with low socio economic status and who were engaged in labor work and also the person who maintained very poor hygiene is most of them wore the same dress for many days. Spores and vegetative forms in the unwashed cloth cause reinfection. This study shows that the fungal infection for the year 2006 was 12%. The Dermatophyte infection was 8% and the remaining fungal infections were 4%. This leads to increased incidence of superficial fungal infection in lower socio economic group⁴.

Superficial fungal infections occur in the outermost layers of the skin, nails, hair, and mucous membranes.

1.1 TYPES OF FUNGAL INFECTIONS

1.1.1 TOPICAL/SUPERFICIAL DISEASE CAUSED BY FUNGAL PATHOGENS

Superficial fungal infections occur in the outermost layers of the skin, nails, hair, and mucous membranes⁵.

1.1.1.1 Dermatophytosis

Dermatophyte fungi are organisms that digest keratin⁶. Dermatophytes infect the stratum corneum of the epidermis and keratinized tissues derived from it, such as hair or nail. *Trichophyton spp.*, *Microsporum spp.*, and *Epidermophyton spp.* are responsible for most of the superficial fungal infections, although the causative agents can be some yeast and some non-dermatophyte molds.

1.1.1.2 Tinea Pedis

Tinea pedis is a dermatophyte infection of the foot, affecting particularly the toes and sole caused mainly by *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* pathogens⁷. This infection affects 15–30% of the population and is the most common dermatophyte fungal disease that occurs in man. Individuals with tinea pedis may be susceptible to secondary bacterial infection with, for example, Group A streptococcus.

1.1.1.3 Tinea Corporis

Two major causative organisms causing tinea corporis are *T. rubrum*, *T. mentagrophytes* affecting neck, trunk, and the extremities. The classic tinea corporis lesion is a sharply defined circular lesion with erythema, scaling, and small blisters or pustules at the border. The lesion is usually <5 cm in diameter. The fungus is often transmitted from domestic animals, such as cats, dogs, hamsters, and guinea pigs to humans⁸.

1.1.1.4 Tinea Capitis

The predominant causative agent of this infection is *Trichophyton tonsurans* and mainly causes disease in childhood, presenting with alopecia and scaling on the scalp⁹.

1.1.1.5 Tinea Unguium or Onychomycosis¹⁰ (Nails)

T. rubrum and *T. mentagrophytes* dermatophytes are the principal causes of onychomycosis, accounting approximately for 90% of toenail infections and 50% of fingernail infections.

1.1.1.6 Superficial Candidiasis

Superficial candidiasis infections are usually caused by *Candida albicans*, and this organism is a common commensal in the mouth, vagina, and gastrointestinal tract in healthy individuals¹¹. The prevalence of carriage is greater in hospitalized patients and those who are immuno-compromised.

1.1.1.7 Oropharyngeal Candidiasis (Oral Thrush)

It has typical symptoms and signs of soreness and white patches on an erythematous background (plaque type)¹⁰. An erythematous variety exists; this does not have plaques, but sore areas of erythema are typical. Acute or chronic infection can occur in immuno-compromised individuals. Other predisposing factors include antibiotic therapy and dentures.

1.1.1.8 Vaginal Candidiasis (Vaginal Thrush)

Vaginal candidiasis is a common infection, with clinical appearances similar to those of oropharyngeal disease, plus vaginal discharge. Pruritus can also occur, and recurrent episodes are common. Women with vaginal thrush seldom have underlying predisposing factors¹².

1.1.1.9 Candidiasis of The Skin

Candidiasis of the skin is often confined to body folds, including the inter-digital spaces of the hands or feet. Typically, small satellite pustules lie distal to the periphery of the rim of the rash. Chronic paronychia (nail fold infections) can be caused by *Candida*¹³.

1.1.1.10 Malassezia Infection¹⁴

Malassezia spp. are common surface commensals of greasy skin includes scalp, chest, and they are associated with pityriasis versicolor, seborrheic dermatitis, and folliculitis.

1.1.1.11 Pityriasis Versicolor¹⁴

Pityriasis versicolor is a scaly, hypo- or hyper-pigmented rash on the trunk which is found common in tropical regions and the patches can resemble vitiligo, but the presence of scaling is typical.

1.1.1.12 Seborrheic Dermatitis¹⁴

Seborrheic dermatitis is a common scaly condition affecting the face, the front of the chest, and the scalp. Severe seborrheic dermatitis is particularly common in patients with AIDS or chronic neurological Parkinson's disease.

1.1.1.13 Malassezia Folliculitis¹⁴

Malassezia folliculitis is an itchy, follicular rash on the upper back and shoulders that can resemble acne.

1.1.1.14 Subcutaneous Infection¹⁵

Although subcutaneous mycoses can disseminate, they are usually limited to the dermis and subcutaneous tissues.

1.1.1.15 Sporotrichosis

Sporotrichosis is caused by the dimorphic fungus *Sporothrix schenckii* and is the most prevalent subcutaneous infection¹⁶. The fungus is found in soil, vegetation and usually causes disease in farmers or gardeners, especially those who tend roses. It is a localized cutaneous or subcutaneous lesion, which may spread via the lymphatic system and form further lesions. Lymphocutaneous sporotrichosis is a non-life-threatening disease.

1.1.1.16 Chromoblastomycosis

Chromoblastomycosis is a chronic cutaneous or subcutaneous fungal infection caused by members¹⁷ of the Dematiaceae family including *Fonsecaea pedrosoi*, *Cladosporium carrionii*, *Fonsecaea compacta*, *Phialophora verrucosa*, and *Rhinocladiella aquaspersa* and found in wood, vegetable debris, and soil. Symptoms are raised, crusted lesions of the skin.

1.1.1.17 Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis is a rare syndrome consisting of chronic infection of mucous membranes usually by *C. albicans*, which may extend to the skin and nails¹⁸. The condition is associated with impaired cell-mediated responses to *Candida*, although the underlying defect remains poorly understood. Various manifestations including white fissured lesions; hyperkeratotic, granulomatous and vegetating lesions, autosomal recessive trait associated with endocrine disorders, for example, hypoparathyroidism.

1.1.2 SYSTEMIC FUNGAL INFECTIONS¹⁹

Systemic fungal infections can be divided into two distinct groups: The endemic or dimorphic mycoses. These infections are caused by true pathogenic fungi as compared with the opportunistic mold and yeast infections that are saprophytes, which only will invade an immuno-compromised host. Such infections are life-threatening and are associated with high rates of death. Solid organ transplant recipients who take immunosuppressive medications to limit the risk of rejection also have an increased susceptibility to systemic fungal infections.

1.1.3 OPPORTUNISTIC PATHOGENS²⁰

1.1.3.1 Invasive Candidiasis

At present, *Candida* spp. rank as the fourth most frequent cause of nosocomial bloodstream infections. Nosocomial candidiasis may be either endogenous which is acquired through previous colonization of the mouth, gastrointestinal tract, vagina or skin or by exogenous which is acquired by cross-infection from another patient or healthcare worker. *C. albicans* is the most frequently isolated species, causing 48–60% of bloodstream fungal infections²¹. However, a change in the pattern of *Candida*-related disease has been resulting in

the emergence of a number of important non-albicans *Candida* spp., such as *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata*. This epidemiological change has major clinical implications by some non-albicans *Candida* spp. and has higher complication and death rates than *C. albicans* infections, and some species are resistant to antifungal agents.

1.1.3.2 Invasive Aspergillosis

Aspergillus spp. is ubiquitous, occurring most frequently in soil, water, and decaying vegetation. Most *Aspergillus* infections are acquired through the respiratory tract inhalation and are associated with hospital construction work or contaminated ventilation systems. Infections may also be acquired from plants or certain foods such as pepper. Sign and symptoms include: Unremitting fever and pulmonary infiltrates during antibiotic therapy, chest pain, pleural rub, pleural effusion, and hemoptysis. Computed tomography scan shows characteristic halo and air crescent signs while radiography reveals single or multifocal lesions.

1.1.3.3 Cryptococcus

Cryptococcal infection usually results from the inhalation of *Cryptococcus neoformans*, which is found primarily in soil contaminated by pigeon or chicken excreta. *Cryptococcus* has a particular affinity for the central nervous system, resulting in Cryptococcal meningitis, and is one of the most significant life-threatening fungal infections associated with HIV²². Cryptococcal infection may also be seen in non-immuno-compromised individuals and patients with impaired cell-mediated immunity, for example, that undergoing solid organ transplantation.

1.1.3.4 Zygomycosis²³

Fungal infections from the class Mucorales (*Mucor*, *Absidia*, and *Rhizopus*) are seen increasingly in immune-compromised hosts. Mucorales infections are typically an airborne disease, initiated in the upper or lower airways and have clinical symptoms similar to those of aspergillosis.

1.1.4 OTHER INVASIVE INFECTIONS²⁴

Rarer opportunistic pathogens that have emerged during recent years include *Penicillium marneffe*, *Fusarium* spp., *Malassezia* spp., *Trichosporon* spp.,

Saccharomyces cerevisiae, and *Blastoschizomyces capitatus*. Invasive infection by *Malassezia furfur*, a commensal yeast normally associated with the superficial fungal infection. *Tinea versicolor* has also increased in frequency in recent years and is associated with parenteral nutrition.

1.1.4.1 Endemic Pathogens

Systemic endemic mycoses include a group of dimorphic fungi that are found in distinct geographical regions.

1.1.4.2 Blastomycosis

Blastomycosis is the dimorphic fungi caused by the pathogens *Blastomyces dermatitidis* and *Blastomyces gilchristii*, which are found in humid soil containing decaying vegetation or decomposed wood and are associated with freshwater drainage basins. It is reported mainly in North America and in Africa but occasionally has also been reported in Central and South America, Mexico, India, and the Middle East.

1.1.4.3 Histoplasmosis

Histoplasmosis caused by the dimorphic fungus *Histoplasma capsulatum* is found worldwide, but particularly in North, Central, and South America. Depending on the immune status of the host and the infectious dose, the clinical manifestations vary. In immunocompetent persons, the disease is usually asymptomatic or manifests as an acute respiratory illness that is self-limiting, whereas in immunocompromised persons, it can result in severe illness with progressive pulmonary disease or disseminated infection. Symptoms are usually mild, but due to heavy exposure of fungus in individuals may cause fever, chills, headache, myalgia, anorexia, cough, and chest pain.

1.1.4.4 Coccidioidomycosis

It is endemic in the southwestern parts of the USA (California, Arizona, New Mexico, Utah, and Nevada) and parts of Central and South America (Mexico, Brazil, and Argentina) and caused by the dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii*. The most common clinical manifestations are chest pain, cough, fever, weight loss, and fatigue, often associated with dermatological

manifestations including erythema nodosum or erythema multiforme and rheumatological manifestations including myalgia and arthralgia. The disease can also spread from the lungs hematogenously to bones, joints, skin, and the central nervous system.

1.1.4.5 Paracoccidioidomycosis²⁵

Paracoccidioidomycosis is caused by the dimorphic fungi *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*. These are found in certain parts of South America, especially not only in Brazil but also in Argentina, Colombia, Ecuador, Peru, and Venezuela.

1.1.4.6 Penicilliosis²⁶

In Southeast Asia, penicilliosis is now the third most frequently occurring opportunistic infection in HIV-infected patients. Isolated cases have also been reported in western countries caused by *P. marneffei*.

1.2 DRUGS USED FOR TREATMENT OF FUNGAL INFECTIONS

Various drugs are available for the treatments of fungal infections as shown in Table 1.1 are enumerable. In addition to this, drugs are also available in ayurvedic, siddha and unani. Antifungal drugs are also known as an anti-mycotic drug.

1.2.1 ALLOPATHIC ANTIFUNGAL AGENTS

There are several types of antifungal medicines. They come as creams, sprays, solutions, tablets designed to go into the vagina (pessaries), soaps, shampoos, medicines to take by mouth, and injections. Most work by damaging the cell wall of the fungus, which causes the fungal cell to die. The antifungal drugs²⁷ are shown in Figure 1.1

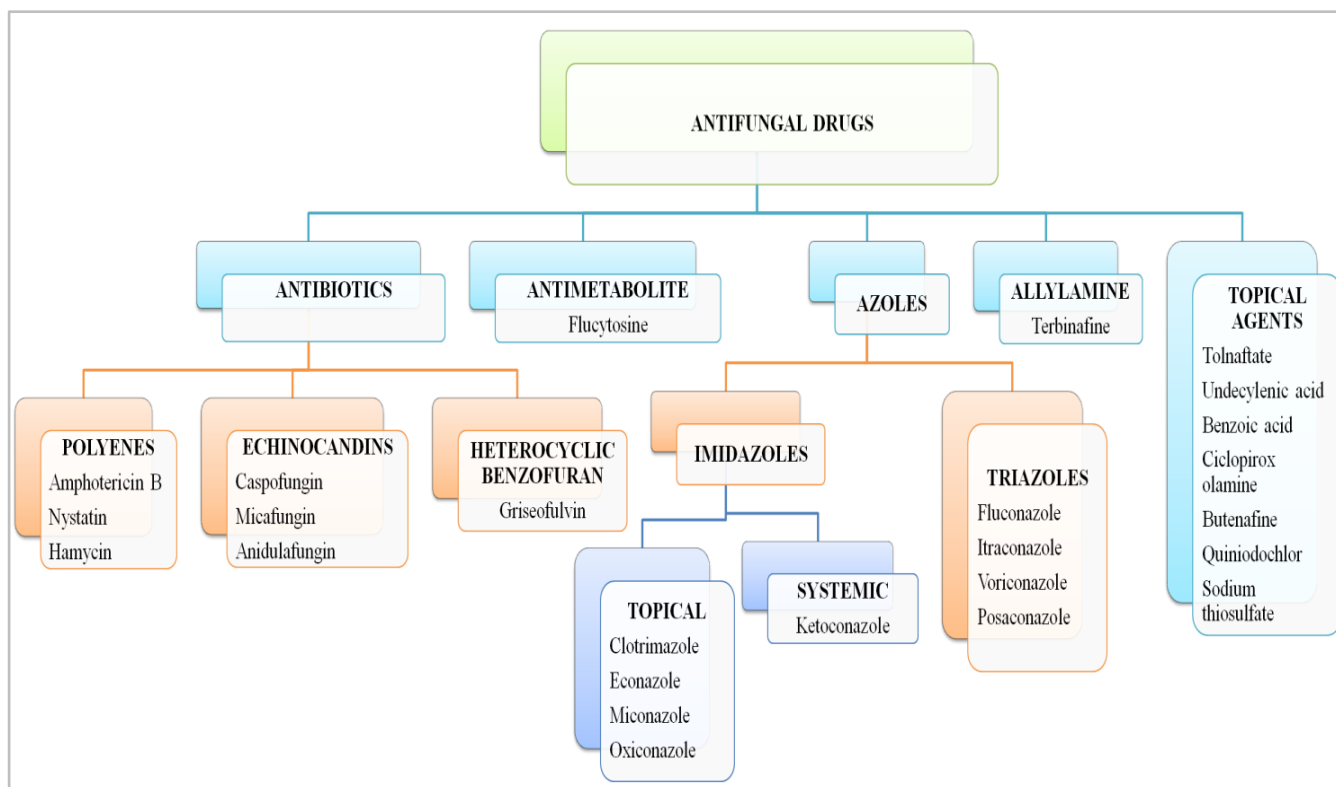


Figure 1.1 Drugs used for treatment of fungal infections

(Tripathi, K. D. 2013)²⁷

1.2.1.1 Allopathic Marketed Preparations

Few examples of marked allopathic drugs are shown in Table 1.1

Table 1.1 Marked allopathic drugs

S.NO	Branded Drug	Generic name
01	Zilzip	Ketoconazole 2% Soap
02	Iptozol	Itraconazole Soap
03	Luliris	Luliconazole 1% Soap
04	Ketoraz	Ketoconazole Soap
05	Kijol	Ketoconazole & Zinc Pyrithione Soap
06	HSLZ	Luliconazole Soap

07	Canesten cream 1%w/w	Clotrimazole
08	Oricon cream 1%w/w	Econazole
09	Lemico cream 2% w/w	Miconazole
10	Lamisil cream	Terbinafine
11	Diflucan tablet 150mg	Fluconazole
12	Daktarin gel 2%w/w	Ketoconazole
13	Nystan Oral Suspension	Nystatin
14	Phosome Injection	Amphotericin

1.2.2 HERBAL ANTIFUNGAL AGENT

Medicinal plants are of great importance to the health of individuals and communities, and their importance lies in the chemical substances that produce a definite physiological action on the human body²⁸. Many of the pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis, and quinine while their purification and quantification makes them more predictable and chemical processing can sometimes modify their effects in desirable ways. Herbal remedies tend to have a more complex and subtle mix of chemicals and can sometimes offer access to drugs or combinations of drugs that the pharmaceutical industry has not yet exploited. These natural compounds formed the basis of discovering modern drugs. Some of the antifungal drugs most recently introduced in clinical practice are echinocandines and sordarines derived from natural products²⁹. Therefore, there is a need to develop new antifungal agents providing new mechanisms of action, with a broad spectrum of antifungal activity, fewer dose-limiting side effects, and economic. This will be beneficial for the pharmaceutical industry when formulated. Herbal formulations always have attracted considerable attention due to their good activity and comparatively lesser side effects when compared to synthetic drugs.

1.2.2.1 Herbal Marketed Preparations^{30,31}

1.2.2.1.1 Hinguliya Manikyarasa

Hinguliya manikyarasa is prepared from purified hingula (cinnabar), gandhak, haritala (yellow orpiment) and juice of palash (flame of the forest) flowers. The herb haritala in this medicine has antimicrobial and antifungal properties against species such as *Staphylococcus aureus* and *Candida albicans*. Consequently, this medicine is useful in the treatment of vaginal yeast infections.

1.2.2.1.2 Dadrughni Vati

It is a tablet-like formulation with chakramarda as its primary constituent. It is one of the best antifungal Ayurvedic medicines and is useful in the management of all types of skin infections including ringworm, and white spots.

1.2.2.1.3 Chandraprabha Vati

This medicine has 42 ingredients, which include shatavari (hundred roots), rock salt, trikatu (a combination of the three acrids – pippali) [long pepper], shunthi [dried ginger] and maricha, triphala, bhringraj, (bhanga) and guggulu (Indian bdellium tree). It is used as a preventive medicine to reduce post-surgical complications in individuals with urogenital diseases. This medicine treats fungal infections of the urinary system and other urogenital problems including dysuria, urinary incontinence and haematuria (blood in the urine). You can take Chandraprabha vati with milk, honey or as per your doctor's direction.

1.2.2.1.4 Arogyavardhini Vati

Arogyavardhini vati is a well-known and extensively used Ayurvedic medicine indicated in the management of skin diseases. It consists of several ingredients including trivrit (Indian jalap), neem, triphala, abhraka bhasma (calcined preparation of mica) and tamra bhasma (calcined preparation of copper). These ingredients of arogyavardhini vati have pitta virechana (elimination of pitta through faeces), vata anulomana (pacification and regulation of vata) and kapha shamana (pacification of kapha) properties. It also has deepana (hunger-increasing), medohara, pachana (digestive) and tridosha shamaka (tridosha-pacifying) effects on the body.

Arogyavardhini vati helps in the alleviation of aggravated doshas and aids in various purification procedures.

1.2.2.1.5 Kaishora Guggulu

Guggulu (Indian bdellium-tree), guduchi (heart-leaved moonseed) and triphala are the primary constituents of kaishora guggulu. This medicine destroys increased pitta and purifies blood. It treats all types of skin diseases including fungal infections and also enhances the lustre of skin.

1.2.2.1.6 Gandhak Rasayana

Gandhak rasayana is a polyherbal formulation prepared from purified gandhak, cow's milk, and a decoction of twak (cinnamon) ela (cardamom), nagakesara (Indian rose chestnut), triphala, shunthi, juice of guduchi, bhringraj (bhanga), adraka and various other ingredients. This medicine is commonly used in the treatment of fungal infections caused by Candida albicans.

And few others such as Vamana, Lepa, Rasonam, Adraka, Yashtimadhu, Haridra, Neem, Ashwagandha, Tulsi, Vasa can be used for treatment for fungal infection.

1.2.3 DIETARY AND LIFESTYLE CHANGES FOR A PATIENT WITH FUNGAL INFECTIONS³¹

1.2.3.1 Do's

- Wear loose-fitting and clean clothes.
- Maintain good personal hygiene.
- Change your clothes twice a day.

1.2.3.2 Don'ts

- Do not suppress natural urges like the urge to eat, drink, urinate or evacuate the bowels.
- Do not drink chilled or cold water immediately after physical activity or exposure to sunlight.
- Do not eat incompatible food combinations like fish and milk.
- Do not overeat or eat foods that are difficult to digest.

- Do not eat contaminated food items.
- Avoid eating excessively salty or acidic food items.
- Avoid sleeping during the daytime.

The above-mentioned drugs and change in life style can help in reducing the fungal infection caused by various pathogens. Since, skin is the most exposed area to the external environment it gets affected easily. And these damaged skins allow the pathogens to penetrate inside the skins as shown in Figure 1.2³² and to avoid any infectious microorganisms from spreading hygiene plays an important role. Hence, soap plays a major role to maintain the skin hygiene by protecting and to control the microbe growth.

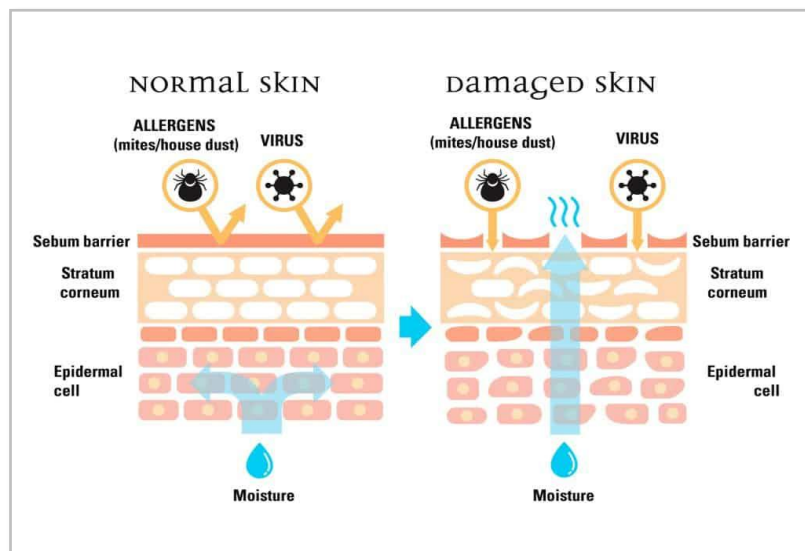


Figure 1.2 Normal skin vs. Damages Skin

(<https://skinlibrary.co.uk/blogs/librarian-edits/skin-barrier>)³²

1.3 INTRODUCTION ON SOAP

Soap has a long history, dating back thousands of years to Ancient Babylon. In the early 2800 BC Archaeologists have discovered soap like material in historic clay cylinders dating from this period. They used to make soap by boiling the fats with ashes. From the prehistoric time personal cleaning has been done regularly to scrap of the oil and dirt. From 17th century that cleanliness and bathing started and come into fashion in Europe³³. From 19th century soap were taxed very heavily in many countries. Soaps were produced in large scale from 1791 by a French

chemist, Nicholas Leblanc. There was a scarcity of animal and vegetable fats and oils used in soap production during World War I and again during World War II. Chemists were forced to use alternative raw materials, which were "synthesised" into chemicals with similar properties. Since, then most commercial soaps contain chemicals that are harmful to the skin. Hence, using a natural herbal soap can be a good alternative like back days. The history of soap³⁴ is shown in Figure 1.3.

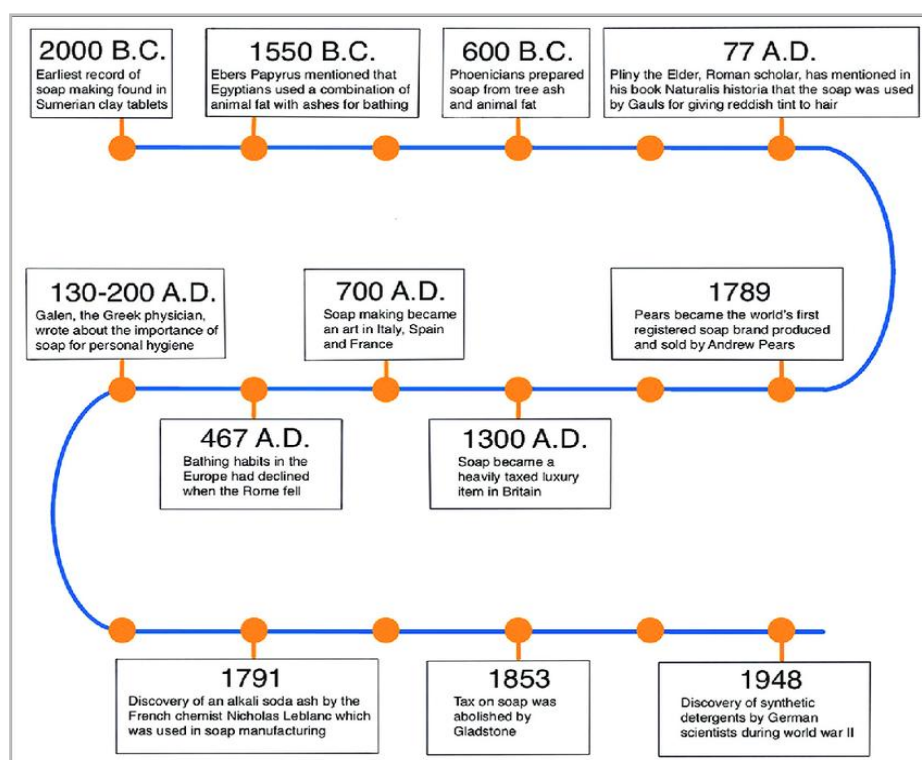


Figure 1.3 Time line of history of soap

(Sakkaravarthi, Vinupriya, 2022)³⁴

Herbal soaps have been found to be extremely beneficial to the skin. The herbs used in these soaps have therapeutic and healing properties that provide specific skin benefits such as nourishment, strength, healing, and moisturising³⁵. The exterior surface of the body is covered by the skin/cutaneous membrane. In terms of weight and surface area, it is the largest organ in the body. The skin's functions include controlling body temperature, serving as a blood reservoir, and providing protection from the environment³⁶. Since, skin is the most exposed area to the environment it's more prone to skin infection and to avoid any infectious microorganisms from spreading hygiene plays an important role. Hence, soap plays a major role to maintain the skin hygiene by protecting and to control the microbe

growth. A pharmaceutical or medication that contains antibacterial and antifungal ingredients is known as an "herbal soap preparation"³⁷. It's made up of plant parts including leaves, stems, roots, and fruits, and it's used to treat damage, disease, and keep people healthy. (Figure1.4) shows few examples of antifungal herbal soap which are available in market.



Figure 1.4 Antifungal herbal soaps

Although wide progress has been made in recent decades in medicine, fungal infections are still an unsolved health problem. It is mainly due to the fact that some of the available antifungal drugs cause resistance. The plant kingdom is a rich source of medicinal preparations that offer a wide chemical diversity, making it of huge potential for new drug development. Phytochemistry of various plant species has indicated that the phytochemicals could be a better source of medicine as compared to synthetically produced drugs. Researchers over the last years have developed a variety of chemical structures with antifungal activity based on natural compounds which are in the process of design and development. Thus, the plant kingdom holds a lot of potential which further needs to be explored in depth. Hence, in the present study; *Macroptilium Lathyroides* and *Acalypha Indica* were selected from the plant kingdom to prepare the antifungal herbal soaps.

CHAPTER- 2

LITERATURE SURVEY

2.1 LITERATURE REVIEW ON *ACALYPHA INDICA*

G.Kamarajan et al., (2022) have synthesized nanoparticles of Zinc oxide (ZnO) using aqueous leaf extract of *Acalypha indica*. Their photocatalyst degradation and antibacterial properties were also measured. The nanoparticles were evaluated/characterized by UV–Visible, XRD, FT-IR, SEM with EDAX, DLS, ZE and PL. They have observed four bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The nanoparticles which were synthesized by active ingredient exhibited good antimicrobial activity against pathogenic bacteria.

[A.Chinnathambi et al., \(2021\)](#) have synthesized nanoparticles of Titanium dioxide (TiO₂) using *Acalypha indica* as a source. The nanoparticles were characterized by FESEM, XRD, UV and FTIR which described the morphology, crystalline nature, and functional group of TiO₂ nanoparticles and indeed TiO₂ had a photocatalytic activity which was analyzed with the degradation of methylene blue dye. They have four parameters in dye degradation such as dosage of nanoparticles, pH, time, and dye concentration were optimized and it was considered for the removal of pollutants by physical and chemical methods which were not effective.

G Shanthini Nachiar et al., (2021) have worked on *Tridax procumbens* and *Acalypha indica* for their antibacterial potency. Chlorhexidine is commonly known for its disinfectant and antiseptic property which is available in many dosage forms. The emerging resistance towards chlorhexidine and other antibiotics such as, clindamycin, gentamycin, bacitracin, polymyxin etc. So, in this review they have assessed the methods for detection of reduced susceptibility to the following substance and the prevalence of co-resistance to other antimicrobial agents. They have observed that these species are found to have good antimicrobial activities especially against *E. coli*, *B. subtilis*, *S. aureus*, and other *streptococcus species* and also the combined extract of the above mentioned resistant developed antibiotic and antiseptics.

Sudhakar Chekuri et al., (2020) have done review on the information of *Acalypha indica* from several aspects such as phyto-chemical content, ethno medicinal practice and pharmacological activities, from entire regions. This plant is applicable for treatment depending on the therapeutic activities and the preferred part of the plant for ethno medicinal practice is its leaves and the root. In these studies, 24 pharmacological activities were identified with some positive results and the most potential therapeutic treatments are anti-cancer, anti-inflammatory, anti-helminthic, antibacterial, anti-diabetes, anti-hyperlipidemic, anti-obesity, and anti-venom and wound healing properties.

NK Yeng et al., (2019) have taken 15 rats on each rat wound was created, on one side it was used as test and other control. Three rats, were euthanized every 3 days, which was on day 3, 6, 9, 12 and 15 after wounding. The body weight of the rats was recorded and skin samples were obtained for histopathology. The Parameters such as epithelialization, angiogenesis, number of PMNLs and macrophages, fibroblast and collagen deposition were used in evaluating the wound healing effect, and the results showed increasing of the body weight of the rats indicated that rats were not affected by the wound induction. They have observed fibroblastic activity, collagen deposition and angiogenic activity were also much higher in the treated wound. Finally, it was conclusion as *Acalypha indica* extract has good wound healing effect.

MS Kumar et al., (2019) have synthesize nanoparticles of Zinc sulphide (ZnS) using *acalypha indica* and *curcuma longa* plant extract using chemical co-precipitation method and the structural, morphological, element composition of biosynthesis ZnS NPs was characterized by XRD, SEM and EDAX with UV Visible spectroscopy. From this investigation formation of inhibition zones clearly shows biosynthesized ZnS NPs have high antimicrobial activity against tested organisms, especially *curcuma longa* plant extract mediated ZnS NPs was formed maximum inhibition against all the tested microorganism. And the nanoparticles synthesized exhibited presence of biomolecular from plant extracts.

Nor Syahiran Zahidin et al., (2017) have reviewed, the information of *Acalypha indica* studies from several aspects such as phytochemical content, pharmacological activities, and ethnomedicinal practice from entire regions and the consumption of *Acalypha indica* as an ethnomedicinal herb has been discussed and associated

with relevant pharmacological studies and phytochemical contents. The whole plant is applicable for treatment depending on the therapeutic activities such as Estrogenic activity, Neuroprotective activity, Diuretic, Anti-viral, Anti-inflammatory, Antioxidant, anti-helminthic, anti-inflammatory, antibacterial, anti-cancer, anti-diabetes, anti-hyperlipidemic, anti-obesity, and it acts as an anti-venom agents is hepatoprotective, it can be used to treat hypoxia and has wound healing properties.

2.2 LITERATURE REVIEW ON *MACROPTILIUM LATHYROIDES*

A.M.Idris *et al.*,(2003) *Macroptilium lathyroides* is a perennial weed which has been found to effect begomovirus in the Caribbean, MaMPRV and MaYMFV has been cloned, characterized and their DNA was modified. And was found out that the two viruses were similar but only MaYMFV infected (*Malva parviflora*) and soya bean these results indicate that MaMPRV and MaYMFV are new, previously undesirabed species of BGYMV group. Both theses can be used to treat the infection that occurs on bean.

Bhagirath S. Chauhan *et al.*, (2017) have worked on bushbean to determine the light, temperature, salt, and water trees, seed burial depth and amount of rice residue on seed germination and seeding emergence and they found out that *Macroptilium lathyroides* is helpful for management in rice field.

Laise S. Passos *et al.*, (2017) have isolated begomovirus which infectes of *Macroptilium Lathyroides* showing the symptoms yellow mosaic and DNA A and B were cloned using rolling circle amplification and subsequently sequenced this new genus taxonomical characterized shows that it is effective to kill begomovirus.

Leoncio M. desousa *et al.*, (2012) have worked on investigation of the streams and branches of *Macroptilium Lathyroides* they have isolated a fixer of beta sitosterol and stigmasterol the NMR spectra shows *Macroptilium Lathyroides* has antibacterial effect.

Mubarak sidding Hamad *et al.*, (2021) have work on finding a new species that is *Macropitillium Lathyroids* in the area flora of Sudan and was absorbed at first in Tutti in land the morphogical description of the plant was identified. This plant is known for nitrogen fixed and thus useful for soil amendement

Mamadou sidybe et al., (2022) have found *Macropitillium Lathyroid* in Senegal location, west Africa and its morphological characteristic was studied and they reported that these species could partly solve the problem of livestock feed which is a bottle neck for livestock in senegal and also it is effective fixing nitrogen in soil.

2.3 LITERATURE REVIEW ON ANTIMICROBIAL SOAP

R. Jithendran et al., (2021) have done a comparative study of various herbal plant such as in Combination 1- *cassia fistula*, *ficus religiosa*, *milletiapinnota* Which is an antimicrobial soap for the nascominal infection. In combination 2- *H.indicus*(aanatamool), *s.lappa*(kushta), *c.rotundus*(mustaka) Which is an herbal soap used for tyrosinase inhibiting property. In Combination 3- *Curcuma longa*, *azadirachta indica*, *allium sativum* Which is an antimicrobial activity is carried out in the organism of *staphylococcus aures*. In Combination 4- *azadirachta indica*, *ocimum tenuiflorum* and *sapindus mukorossi* there is a presence of zone of inhibition. In Combination 5- *vetiveriazizanioides*, *vitex negundo*, *neem*, *tulsi* have observed that there is presence of zone inhibited and various parameters are carried out. And finally, found that combination 5 had good antibacterial activity with improve the skin tone.

Munde Govind Anant et al.,(2021) have synthesized herbal soap, which was formulated by using leaf of neem, aloe vera, tulshi, vit-c, tocopheryl acetate. Ayurvedic cosmetics are very helpful and produce any side effects. Neem has more medicinal properties and exhibit anti-inflammatory, anti-hyperglycemic, antiulcer, anti-malarial, antifungal, antibacterial, anti-mutagenic and anti-carcinogenic property. Aloe plant is used to treat burn the skin conditions psoriasis and even acne, the antioxidant properties of tocopheryl acetate can help to prevent damage to skin caused by free radicals from UV exposure and tulshi give many benefits for the skin like deep clean. So, this soap preparation provides medicinal properties like antibacterial, antifungal activity which can be useful to treat various skin problems.

A.A. Warra et al., (2020) have studied to determine the antibacterial activity of some selected seed oil from plant for soap formulation by using cold saponification process and the selected seed oils are baobab oil, canary oil, castor beans oil, wild grapes, cottonseed oil, onion oil, and calabash seed oil with sodium hydroxide. The sodium hydroxide solution was added to each seed oils in ratio 1:1 v/v and activities of the

soap against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Salmonella typhi* were investigated. The results revealed that soap formulated with onion seed oil possessed the highest activity against all bacterial strains.

Rutuja R. Shah et al., (2020) have determined the antifungal activity of garlic oil in this type of soap is typically effective against athlete's foot and jock itch and it may also be useful for treating other non-fungal skin conditions such as psoriasis and eczema. In present work antifungal soap of garlic oil is formulated by using these ingredients such as soap base, garlic oil, glycerin, methyl paraben, perfume and distilled water. They performed Microbial study, carried out using microorganism such as *proteus vulgaris*, *bacillus*, *klebsiella pneumoniae*, *staphylococcus aureus*, *pseudomonas aeruginosa*, for measuring the effectiveness of an antimicrobial agent against fungi grown in culture, the microorganism of the interest was swabbed uniformly across a culture plate. Then soap of 5 x 5 mm was placed on the surface of the agar and the plates were placed in the incubator for 24 hrs at 30°C. Hence, they have concluded that the herbal soap formulation exhibited a good antifungal effect and various physicochemical parameters for which good characteristics were observed.

Thirunavukkarasu et al., (2020) have done a comparative study on Ultrasound-assisted extract of *Acalypha indica* was used to prepare the green soap formulation by using the components like coconut oil (A, 34–56%), soybean oil (B, 19–31%), castor oil (C, 14–23%), sunflower oil (D, 4–6%), and olive oil (E, 5–9%). Linear regression models were proposed to predict the responses, i.e., hardness (Y_1), iodine (Y_2), and iodine number saponification (INS) (Y_3), and validated with a high degree of statistical accuracy ($F_{cal} > F_{tab}$; $df = 4$, $p < 0.0001$; $R^2 > 0.9950$). In soap formulation were quite comparable concerning the standard soap specifications and antifungal activity was determined by using agar dilution method.

Rakesh K. Sindhu et al., (2019) have formulated and developed the polyherbal hygienic soap by using cold process method and evaluate antimicrobial potential by agar well diffusion method and soap was prepared by using coconut oil, castor oil, Neem oil, Mentha oil, rose petals extract and NaOH (lye) and the different extracts were incorporated into the basic saponification reaction. The herbal formulation was prepared then evaluation for the analysis of pH, moisture content, foaming index, foam retention time, saponification, TFM determination, ethanol soluble matter and

antimicrobial activity using different concentration of soap solution comparing with standard. Hence, evaluation tests showed that the herbal soap has satisfactory antimicrobial results as compared to standard antibiotic.

MV Anoop et al., (2015) have done a comparative study on neem oil and nalpamaradhi oil is to explore its antimicrobial properties to formulate an herbal bath soap and Physicochemical parameters such as color, odour, texture, pH, TFM, moisture content, hardness and foam test were evaluated; these parameters will serve as standardization of the herbal soap. The soap was prepared through cold saponification method by standard procedure using neem oil, nalpamaradhi oil and alkali (Sodium Hydroxide). Hence, evaluation of antimicrobial activity such as determination of antibacterial (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* and *Escherichia coli*) and antifungal activity (*Candida albicans* and *Aspergillus niger*) were performed on the formulated herbal soap and the result shows that the soap has got considerably good antimicrobial property.

CHAPTER- 3

AIM AND SCOPE OF WORK

3.1 AIM:

The aim of the study is to formulate and evaluate antifungal herbal soap using natural ingredient by melt and pour method.

3.2 SCOPE OF WORK:

- To prepare the plant extract with ethanol by maceration technique.
- To perform phytochemical analysis of plant extraction and to study various test such as; test for alkaloids, test for carbohydrates, test for terpenoids, test for phenols, test for saponins, test for flavanoids and test for amino acids.
- To formulate herbal soap for the prepared extract of *Acalypha Indica* and *Macroptilium Lathyroides*. By using melt and pour method.
- To perform various evaluation parameter such as; organoleptic evaluation, physical evaluation, measurement of pH, foam retention, foam height, determination of percentage of free alkali, alcohol insoluble matter, total fatty matter, moisture content, saponification, foam forming ability, and skin irritation test
- To perform antifungal activity by using well diffusion method. Samples were prepared of bacterial inoculums.
- To perform the comparative study with already available marketed soaps.

CHAPTER- 4

EXPERIMENTAL WORK

4.1. MATERIALS:

4.1.1. *MACROPTILIUM LATHYROIDES*⁶¹:

Macroptilium Lathyroides is an herbaceous annual or short-lived perennial growing up to 1 meter high, it is native to the tropical and subtropical areas. Its taxonomical classification is shown in Table 4.1 and its image is shown in Figure 4.1.



Figure 4.1

Table 4.1 Taxonomical Classifications of *Macroptilium Lathyroides*:

Biological name	<i>Macroptilium lathyroides</i>
Taxonomical classification	
Kingdom	Plantae
Class	Equisetopsida C. Agardh
Family	Fabaceae
Subfamily	Faboideae
Order	Fabales

Genus	<i>Macroptilium</i>
Species	<i>M. lathyroides</i>
Commonly name	phasey bean, wild bean, One-leaf clover, Cow pea (English) Kattu avara (Tamil)
Distribution	Andhra Pradesh: Chittoor district Odisha: Cuttack district Tamil Nadu: Kanchipuram (Changalpattu-CGP) district

4.1.1.1 Geographical Source:

Macroptilium lathyroides originated from tropical America, South America, India, Australia, Africa and the southern.

4.1.1.2 Collectivation And Collection:

Macroptilium lathyroides is found from low elevations in the subtropics up to elevations of 2,000 metres in the tropics. It is adapted to a wide mean annual rainfall range from 500 - 3,000 mm. The optimum day/night temperature for growth is 35/20°C. The plant is adapted to acid and alkaline soils, and a wide range of soil textures from sand (given reliable rainfall) to heavy clay. It grows well in a freely draining soil, but is also tolerant of water logging and poor drainage and frequently grows in drains along the edges of roads. Prefers a pH in the range 6 - 7, but can tolerate 5 – 8. Although it has become widely naturalized, it is rarely considered a serious weed. The plant survives drought by shedding its seed, which can lie dormant in the soil until wet weather returns. This species has a symbiotic relationship with certain soil bacteria; these bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby.

4.1.1.3 Macroscopical Characters:

- **Leave Colour:** Greenish brown
- **Flower Colour:** Blood Red

- **Odour:** Characteristic and admissible odor
- **Taste:** Bitter

Other Features:

- **Leaves:** Compound, alternate, trifoliate with elliptical leaflets twice as long as wide. They are carried by a finely pubescent petiole. At the base, there are two striated stipules. The terminal leaflet is far from the lateral leaflets. The three leaflets are held by a very short pedicel, with two very small stipules at its base. The laminas of the leaflets are narrowly elliptic or narrowly oval.
- **Stem:** Stem is cylindrical, solid, and glabrous to finely hairy.
- **Flower:** Papilionaceous flower is pink or crimson. Calyx is tubular with 5 lines of which one is larger.
- **Fruit:** The fruit is a long linear pod, cylindrical, and perpendicular to the axis of the inflorescence. It ends in a tapered beak. It is covered with a short pubescence.

4.1.2. *ACALYPHA INDICA*⁴¹:

Acalypha indica is an annual to sometimes short-lived perennial herb that usually grows up to 1.5 metre tall. It is an important medicinal plant in the Indian Ocean islands as well as in India. Its taxonomical classification is shown in Table 4.2 and its image is shown in Figure 4.2.



Figure 4.2

Table 4.2 Taxonomical Classification of *Acalypha Indica*:

Biological name	<i>Acalypha indica</i>
Taxonomical classification	
Kingdom	Plantae
Class	Dicotyledons
Family	Euphorbiaceae
Subfamily	Acalyphinae
Order	Malpighiales
Genus	<i>Acalypha</i>
Species	<i>A. indica</i>
Commonly name	Indian acalypha, Indian nettle, Kuppameni
Distribution	Throughout the Old-World tropics

4.1.2.1 Geographical Source:

Acalypha indica is a common weed found in Asia including India, Pakistan, Yemen, Sri Lanka.

4.1.2.2 Collectivation and Collection:

Acalypha indica is well distributed in Tropical areas of Africa and South America. It is an annual herb, about 80 cm in height with its predominant habitant being waste places or fields. This plant based medicinal compounds are not only used for primary health care in rural areas of developing countries, but also rural areas of developed countries as well, where modern medicines are predominantly used.

The leaves are 2.5-7.5cm long and 2-2.5cm broad. The shape of the leaves is said to be ovate or rhomboid and ends as acute or sub-obtuse. The leaves have crenate-serrate margin.

4.1.2.3 Macroscopical Characters:

- **Colour:** Greenish brown
- **Odour:** Characteristic and admissible odor
- **Taste:** Slightly bitter
- **Leaves Size:** Length 1.2-6.5; Diameter 1cm-4cm

Other features:

- **Leaves:** Alternate, oblate, rhomboid-ovate, sometimes obovate, subacute or obtuse at apex, cuneate or tapering at base, serrate only in the upper part along the margins, dark-green above, pale-green below, glabrous or thinly hairy, petioles 3-8cm long, hairy, stipules small.
- **Flowers:** Male flowers minute, pale-greenish-yellow, condensed in the upper part, followed by a tuft of sterile flowers. Females lax, in the lower half, scattered, 3-5 leaf, large, dentate, many-nerved bracts. Sepals 4, petals 0, stamens 8, filaments free, ovary 3-celled, styles filiform.
- **Fruits:** Capsules 1-2mm in diameter, green pubescent, Dehiscent, Concealed in the bract.
- **Stem:** Hairs present, Epidermis consists of isodiametric cells. Cortex of 6 to 8 layers with collenchymatous patches alternating with parenchymatous tissue, containing some dark cell contents. Laticiferous cells and laticiferous vessels are present in cortex and pith. Rosette crystals are present in the pith especially.
- **Root:** Transverse section characterised by the presence of deep pale buff coloured wood and comparatively small cortex including cork. Many cortical cells contain starch and rosette crystals of calcium oxalate. The bulk wood mainly made up of tracheids, vessels and wood fibres, medullary rays are composed of 1 to 2 rows of cells, containing starch, fibres with irregular margins and broad or tapering ends. Tracheids have both pitted and scalariform thickening on their wall.
- **Habitat:** Throughout the plains of India. It is a common weed in gardens and waste places.
- **Part Used:** Whole plant.

4.2. METHODS:

4.2.1. COLLECTION OF PLANT SAMPLE:

Macroptilium Lathyroides and *Acalypha Indica* were collected from the ground of **Sathyabama Institute of Science and Technology; Chennai-603119**. And authenticated in Siddha Research Institute, Arumbakkam, Chennai-600106. (Form No: PCOG002-ACF).

4.2.2. PREPARATION OF EXTRACTS:

Leafs, flowers and seeds were dried, powdered and was passed though #40 mesh size. The extraction was done with ethanol as solvent by Maceration technique. The extraction was concentrated and stored for further use⁶⁴. As show in (Figure 4.3 & 4.4).



Collected plants of
Acalypha Indica



Dried leaves of
Acalypha Indica



Dried Powdered of
Acalypha Indica

Figure 4.3 Formulation of Acalypha Indica powder



Collected plants of
Macroptilium Lathyroides



Dried Leaves of
Macroptilium Lathyroides



Dried powdered of
Macroptilium Lathyroides

Figure 4.4 Formulation of *Macroptilium Lathyroides* powder

4.2.3. PHYTOCHEMICAL SCREENING OF PLANT EXTRACTION:

Phytochemicals are regarded as secondary metabolites. The medicinal value of plants depends on numerous active compounds which are helpful in improving the life and treatment of diseases. The ethanol extract of the plant species were analyzed for alkaloids, tannins, phenols, flavonoids, steroids, saponins. Presence of phytochemicals is determined on the basis of standard qualitative test procedures.

4.2.3.1. Test for Alkaloids:

The powdered plant material was boiled with 5 mL of dilute sulphuric acid and filtered. The filtrate was used for the following tests.

a) Mayer's Test

To the filtrate, few drops of Mayer's reagent was added and shaken well. Formation of white precipitate was formed showed the presence of alkaloids.

b) Wager's Test

To the filtrate, few drops of Wagner's reagent was added and shaken well. Formation of reddish-brown precipitate was formed showed the presence of alkaloids.

4.2.3.2. Test for Carbohydrates

a) Benedict's Test

1mL of the analyte sample must be mixed with 2 mL of Benedict's reagent and heated in a boiling water bath for 3 to 5 minutes. The development of a brick-red colored precipitate of cuprous oxide confirms the presence of carbohydrates.

b) Fehling's Test

The substance was boiled with Fehling's solution I and II. Appearance of red color was formed showed the presence of carbohydrates.

4.2.3.3. Test for Terpenoids:

a) Salkowski's Test

5mL of extract was mixed with 2mL chloroform, and concentrated 3mL sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

4.2.3.4. Test for Phenols:

a) Ferric Chloride Test

To the alcoholic extract of the test substance a few drops of 5 % ferric chloride solution was added. Deep blue or black color was formed showed the presence of phenols (Figure 4.5).

4.2.3.5. Test for Saponins:

a) Foam Test

The powdered plant material was shaken vigorously with 5 ml of water. Persistent foam was formed showed the presence of saponins.

4.2.3.6. Test for Flavanoids:

a) Alkaline Reagent Test

The substance was shaken with 5 ml of alcohol and filtered. To the filtrate, 10% sodium hydroxide solution or ammonia was added. Appearance of Dark yellow color was formed showed the presence of flavanoids (Figure 4.5).

b) Lead Acetate Test

The substance was shaken with 5 ml of alcohol and filtered. To the filtrate 1 ml of lead acetate solution was added. Yellow precipitate was formed showed the presence of flavanoids (Figure 4.5).

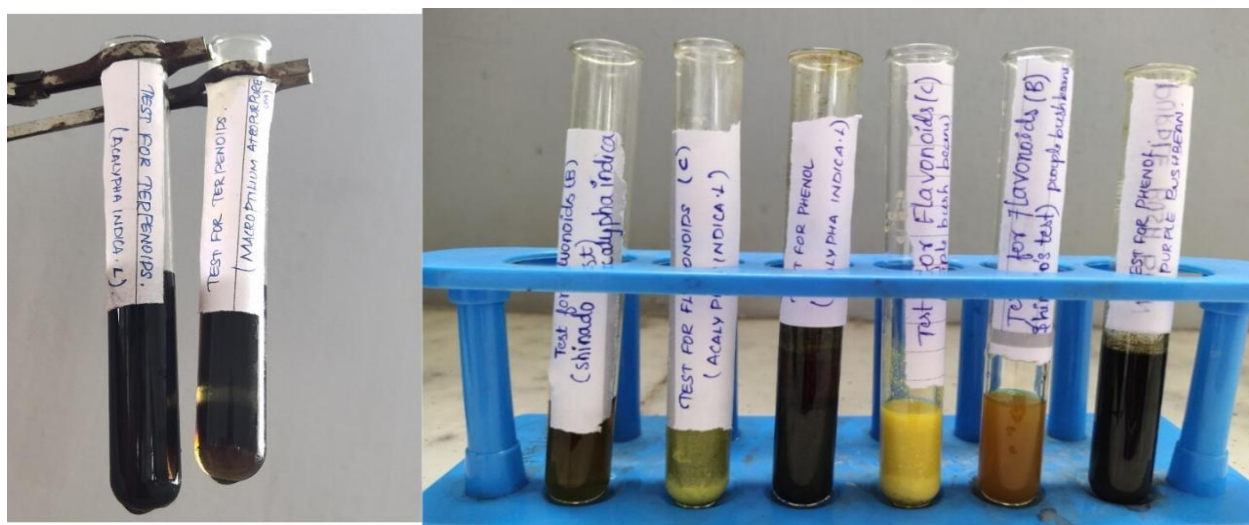


Figure 4.5 Phytochemical Analysis

4.2.3.7 Test for Amino Acids:

a) Ninhydrin Test

3 mL of test solution was warmed with 3 drops of 5% ninhydrin reagent. Formation of purple color was formed showed the presence of amino acids.

Table 4.3 Phytochemical Analysis

Test Name	Phytochemical	<i>Acalypha Indica</i>	<i>Macroptilium Lathyroides</i>
Mayer's test	Alkaloids	+	+
Wager's test	Alkaloids	+	+
Benedict's test	Carbohydrates	-	-
Fehling's test	Carbohydrates	-	-
Salkowski's test	Terpenoids	+	+
Ferric chloride test	Phenols	+	+

Foam test	Saponins	+	-
Alkaline Reagent Test	Flavanoids	+	+
Lead acetate test	Flavanoids	+	+
Ninhydrin test	Amino acids	+	-

4.2.4. FORMULATION OF ANTIFUNGAL HERBAL SOAP:

The Antifungal herbal soap was prepared by melting glycerin soap base double heat method. To this the obtained extract of *Macroptilium lathyroides*, *Acalypha indica* & Lemongrass essential oil were added as shown in Table 4.4. This mixture was continuous agitated until mixture became homogeneous. The mixture was poured into a mould and was allowed to solidify Figure 4.6 shown in the prepared soap was packed in the aluminium cover until further evaluation.



Figure 4.6 Formulation of Antifungal Herbal soap

Table 4.4 Formulation of Herbal Soap

Sr. No	Ingredients		Quantity					
			F1	F2	F3	F4	F5	F6
1.	Glycerine soap base:		20g	20g	20g	20g	20g	20g
	Glycerine	Humectant						
	Coconut oil	Anti-aging & Moisturizer						
	Castor Oil	Lather & Moisturizer						
	Olive oil	Gentle cleansing, Softens & For long lasting of soap						
	Vitamin E	Nourishing & Moisturizer						
	Sodium Hydroxide	Lye						
	Potassium Hydroxide	Lye						
2.	<i>Macroptilium lathyroides</i>	Antifungal	1 mL	2 ML	3 mL	4 mL	5 mL	6 mL
3.	<i>Acalypha indica</i>	Antifungal	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL
4.	Lemongrass essential oil	Perfume	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops

4.2.5. EVALUATIONS:

4.2.5.1. Organoleptic Evaluation⁶²:

Using only our eyes and nose, we evaluated the soaps colour, clarity, and odour against a white background.

4.2.5.2. Physical Evaluations:

The formulated soaps were evaluated for the following properties:

a. Measurement of pH⁶⁵:

1 gm of soap sample was taken & was dissolved in 10mL of water. Using a digital pH meter, the pH of each of the generated formulations was assessed.

b. Foam Retention⁶⁵:

A 100 mL graduated measuring cylinder was filled with 25 mL of the 1% soap solution. The cylinder was shaken ten times with one hand covering it. For four minutes, the volume of foam was measured at one-minute intervals.

c. Foam Height⁶⁵:

In 25 mL of distilled water, 0.5 gm of soap sample was dispersed. The volume was then increased to 50 mL with water in a 100 mL measuring cylinder. Twenty-five strokes were given and left to stand until the aqueous volume reached 50 mL and the foam height above the aqueous volume was measured.

d. Determination of Percentage of Free Alkali⁷⁰:

Measure 10g of soap sample and place it in a beaker with 150 mL of distilled water. Warm the beaker for 30 to 40 minutes to dissolve the soap, then transfer the solution, along with the washings, to the 250 mL conical flask. To reach the 250 mL mark, add more distilled water. If you shake the flask, foam will form, which could lead to incorrect results. Fill the titration flask with 10 mL of the soap solution. In the titration flask, add two drops of phenolphthalein indicator; the color will change to pink. Titrate it against the burette's 0.01M HCl until the solution becomes colorless.

e. Alcohol Insoluble Matter⁶⁵:

A 5 g sample was placed in a conical flask. 50 mL of warm ethanol was added to this, and it was vigorously shaken until the sample was completely dissolved. The solution was filtered through a tared filter paper with 20 mL warm ethanol and dried at 105°C for 1 hour. The weight of dried paper was measured.

f. Total Fatty Matter⁶⁵:

TFM was calculated by reacting soap with acid in hot water and calculating the fatty acids obtained. 10 g of the formulated soap was heated in 150 mL of distilled water. 20 mL of 15% H₂SO₄ was added to this and heated until a clear solution was obtained. The fatty acids on the surface of the resulting solution are solidified by adding 7 g beeswax and heating it again. It was then allowed to cake. The cake was removed, blotted dry, and weighed to determine the TFM using the formula

$$\%TFM = \frac{\text{Weight of the cake (g)} - \text{Weight of the wax(g)}}{\text{Weight of soap(g)}} * 100$$

g. Moisture Content⁶⁵:

5 g soap sample was weighed into a tarred moisture dish after it had been dried at 105°C for 2 hours and chilled to room temperature. Following an hour of heating, successive attainments of constant weight revealed losses of no more than 0.1%.

$$\text{Moisture content}\% = \frac{\text{loss in weight}}{\text{Weight of sample}} * 100$$

h. Saponification^{65, 69}:

Titration of potassium oxide with 0.5N sulfuric acid in the presence of phenolphthalein indicator can be used to determine it. After mixing the sample with 25 mL of an alcoholic KOH solution, it is placed in a steam bath for an hour to react.

Saponification value

$$= (\text{Titre value of blank in ml} - \text{titer value of sample in ml}) \\ * \text{normality of KOH} * \text{equivalent}$$

i. Foam Forming Ability⁶⁵:

To test the ability of the herbal soap to form foam, place about 1.0 g of 50 ml in a 100 mL graduated measuring cylinder. The measuring cylinder was then shaken for about 2-3 minutes before being left for about 10 minutes. After 10 minutes, the foam height was measured.

j. Skin Irritation Test⁶⁵:

Wash your skin thoroughly, then apply the soap sample to your skin and look for any irritation, burning, itching, or any other symptoms.

k. Antifungal Activity: (Well Diffusion Method⁷¹)

Sample Preparation:

1g of soap sample was dissolved in 10 mL of distilled water.

Preparation of Bacterial Inoculums⁶³:

A loopful of fresh test pathogens (*Candida albicans* & *Non albican Candida* sps.) were inoculated in SDB respectively and adjusted to 0.5 McFarland standards.

Procedure:

- Using sterile cotton swab, the test fungal inoculum was seeded on the SDA plates at Sathyabama Institute of Science & Technology Centre for Drug Discovery and Development
- The wells were cutted using corn borer.
- 100 µL of sample were pipetted out in respective wells.
- Control wells were maintained: CC – Carrier Solvent control PC – Positive control
- Incubate the plates at 30 °C for 48 hrs.
- After incubation, zone of inhibition (ZOI) around the wells were measured in diameter (mm).
- All the assays were performed in triplicate.

4.3 COMPARATIVE STUDY:

Two different antifungal soap (i.e.) one herbal bathing soap and medicated soap were purchased from a Retail pharmacy to perform a Comparative study with the formulated herbal soap.

CHAPTER- 5

RESULTS AND DISCUSSION

5.1 RESULTS FOR FORMULATION

5.1.1 RESULT FOR ORGANOLEPTIC STUDY

For the formulation F1&F2 the color and aroma were transparent and very mild; for F3&F4 the color aroma were mild green and pleasant; for F5, the color was dark green and had a pungent odour; and for F6, it was extremely dark green and was pungent.

5.1.2 RESULT FOR PHYSICAL EVALUATIONS

The physical evaluations of herbal soap formulations F1, F2, F3, F4, F5, and F6, include colour, appearance, pH, Foam retention, foam height, percentage of free alkali, alcohol-insoluble matter, total fatty matter, moisture content, foam-forming ability, and skin irritability were measured. For F1, the pH level was 5.6, the foam retention was 3.5 cm, the foam height was 3 cm, the percentage of free alkali was 2%, alcohol insoluble matter was 26%, the total fatty matter was 65.4%, the moisture content was 11.9%, the foam forming ability was 3 cm, and there was no skin irritation. For F2, the pH level was 6.8, the foam retention was 3.0 cm, the foam height was 2.7 cm, the percentage of free alkali was 2.1%, alcohol insoluble matter was 25.9%, the total fatty matter was 65%, the moisture content was 12%, the foam forming ability was 3 cm, and there was no skin irritation. For F3, the pH level was 7.6, the foam retention was 3.2 cm, the foam height was 3.2 cm, the percentage of free alkali was 2%, alcohol insoluble matter was 27%, the total fatty matter was 64.8%, the moisture content was 11.8%, the foam forming ability was 3 cm, and there was no skin irritation. For F4, the pH level was 9.2, the foam retention was 3.0 cm, the foam height was 2.7 cm, the percentage of free alkali was 2.2%, alcohol insoluble matter was 27%, the total fatty matter was 65%, the moisture content was 12.4%, the foam forming ability was 3 cm, and there was no skin irritation. For F5, the pH level was 10.57, the foam retention was 3.5 cm, the foam height was 3 cm, the percentage of free alkali was 2.3%, alcohol insoluble matter was 27.6%, the total fatty matter was 64.7%, the moisture content was 12.3%, the foam forming ability

was 2.9, and there was no skin irritation. For F6, the pH level was 11.19, the foam retention was 3.2 cm, the foam height was 3.2 cm, the percentage of free alkali was 2.3%, alcohol insoluble matter was 26.5%, the total fatty matter was 64.6%, the moisture content was 12.5%, the foam forming ability was 3 cm, and there was no skin irritation. From this F4 have the best physicochemical properties out of the six formulations. Hence, it shows that non-soap substances were not used in the manufactured soaps. The formulated F4 was further sent for antifungal activity in Col.Dr.Jeppiaar Research Park in Sathyabama Institute of Science & Technology. Antifungal activity was studied on *Candida albicans* & *Non albican Candida sps* and showed around 16 – 22 mm in diameter of zone of inhibition as shown in Figure 5.1.

Table 5.1 Physicochemical Parameters of Herbal Soap Formulations

Parameters	F1	F2	F3	F4	F5	F6
Color	Transparent	Transparent	Mild green	Mild green	Dark green	Very dark green
Odour	Very mild	Very mild	Pleasant	Pleasant	Pungent	Pungent
pH	5.6	6.8	7.6	9.2	10.57	11.19
Foam retention (cm)	3.5	3.0	3.2	3.0	3.5	3.2
Foam height (cm)	3	2.7	3.2	2.7	3	3.2
Percentage free alkali (%)	2	2.1	2	2.2	2.3	2.3
Alcohol insoluble matter (%)	26	25.9	27	27	27.6	26.5
Total fatty matter (%)	65.4	65	64.8	65	64.7	64.6

Moisture content (%)	11.9	12	11.8	12.4	12.3	12.5
Foam forming ability(cm)	3	3	2.8	3	2.9	3
Skin irritation	No	No	No	No	No	No

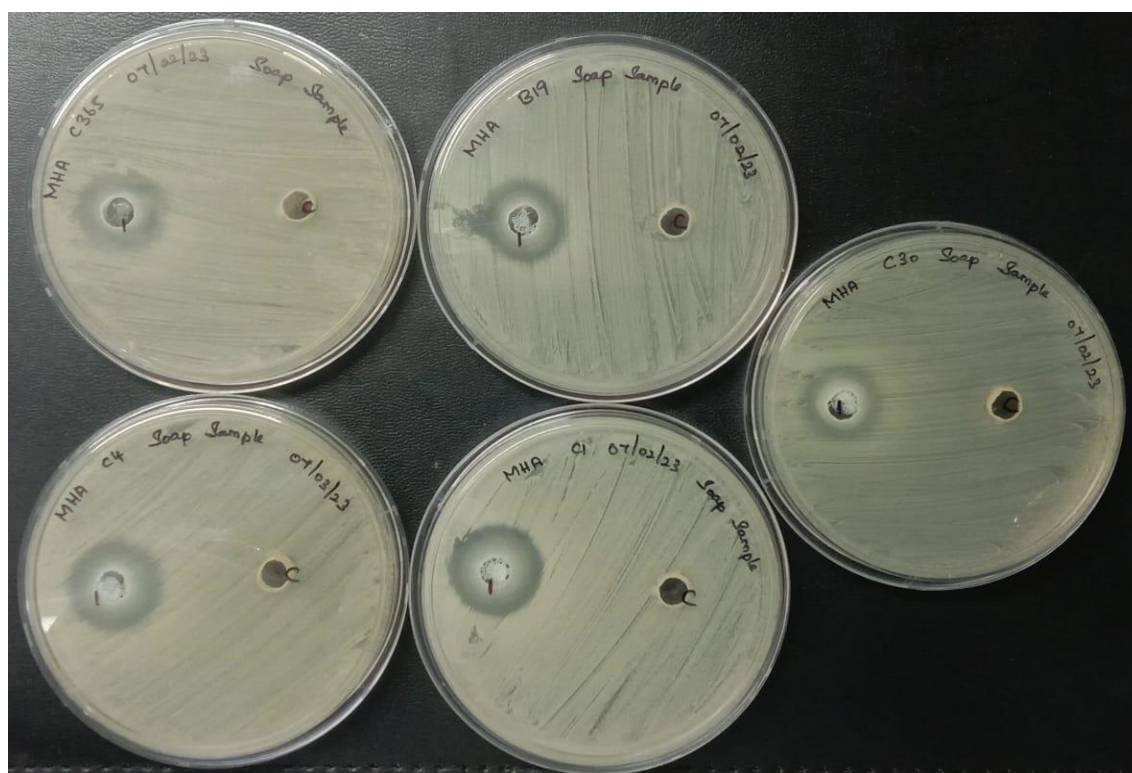


Figure 5.1: Zone of inhibition for F4 formulation

5.2 RESULT FOR COMPARATIVE STUDY

And a comparative study was performed with two marketed drugs—one herbal bathing soap and the other medicated soap. In herbal antifungal bathing soap, the color and aroma were dark green and pleasant; the pH level was 7, the foam retention was 20 min, the foam height was 7.7 cm, the percentage of free alkali was 1.15%, the alcohol insoluble matter was 21.8%, the total fatty matter was 12.11%, the moisture content was 6%, the foam forming ability was 8 cm, and there was no skin irritation. In medicated soap-clotrimazole soap 1% W/W, The colour and aroma were light blue and very mild; the pH level was 9, the foam retention was 5hrs

10min, the foam height was 40.5 cm, the percentage of free alkali was 1.04%, the alcohol insoluble matter was 20%, the total fatty matter was 24.30%, the moisture content was 3.2%, the foam forming ability was 38.4 cm, and there was no skin irritation. Hence, the F4 was the better result shown in Table 5.2.

Table 5.2 Comparative study of F4 VS Marketed soaps

Parameters	F4	Herbal antifungal bathing soap	Medicated soap- Clotrimazole soap 1% W/W
Color	Mild green	Dark green	Light blue
Odour	Pleasant	Pleasant	Very mild
pH	9.2	7	9
Foam retention (min)	11	20	5hrs 10 min
Foam height (cm)	2.7	7.7	40.5
Percentage free alkali (%)	0.5	1.15	1.04
Alcohol insoluble matter (%)	17	21.8	20
Total fatty matter (%)	65	12.11	24.30
Moisture content (%)	12.4	6	3.2
Foam forming ability (cm)	3	8	38.4
Skin irritation	No	No	No

5.3 DISCUSSION:

- The aim of the study was to formulate and evaluate antifungal herbal soap using natural ingredient by melt and pour method.
- Six different formulations were formulated by the addition of extraction to a melted glycerin soap base and their organoleptic, physical, moisture content, total fatty matter, pH, alcohol insoluble matter and foam height properties were assessed.
- For each plant separately a preliminary phytochemical examination was conducted. Alkaloids, carbohydrates, terpenoids, phenols, saponins, flavonoids and amino acids were compared with the study performed by **(G.Kamarajan et al., 2022)** on *Acalypha indica* and in **(PI Ramos and A.Fernandez's 2022)** on *Macroptilium lathyroides*. The results were similar.
- The *Acalypha indica* and *Macroptilium lathyroides* which possessed antifungal data were used to shield the skin against antifungal growth. *Acalypha indica* **(Vasanthan A, senthil Kumar K.L 2022)**⁷¹ worked on formulation and evaluation of antifungal herbal soap⁷¹ using *Acalypha indica* and when compared to F4 formulation. Results from F4 were better.
- From the evaluations it was shown that F4 had the best characteristic feature.
- Since, F4 had the best characteristic feature it was further evaluated for Antifungal activity which showed good result.
- A comparative study was performed with marketed herbal antifungal bathing soap and Medicated soap- Clotrimazole soap 1% W/W. And the formulated F4 showed better result.
- Thus, we can conclude by the saying that the formulate Antifungal herbal soap by using *Acalypha Indica* and *Macroptilium Lathyroides* showed better antifungal activity and futher study such as In -vivo ,scale up can be done.

CHAPTER- 6

SUMMARY AND CONCLUSIONS

6.1 SUMMARY

CHAPTER - 1

Describes about the general introduction of fungal skin infection, its types, various drugs used for treatment of fungal infection, an basic introduction on the soap, and the reason why *Macroptilium Lathyroids* and *Acalypha Indica* were Selected to prepare the antifungal herbal soap.

CHAPTER - 2

Discuss the literature review of the earlier published research works on development and characterizations of antifungal soap. In addition, a special attention was given to review the literature on *Macropitilium Lathyroids* and *Acalypha Indica*.

CHAPTER- 3

Describes the aim and objectives of the research work.

CHAPTER – 4

Describes the materials instrument used and experimental work involved in the research work material like conical flask, beaker, measuring cylinder, water bath, burette, stirrer, heating Mandel etc..It also includes taxonomical classification, geographical source, collectivation, macroscopical characters. Collection of plant sample, preparation of extracts Phytochemical screening of plant extraction such as test for carbohydrates test, test for terpenoids, test for phenols, test for flavonoids, test for amino acids. Chapter 4 also described about the formulation of herbal soap by melt and pour method and its organoleptic evaluation, physical evaluation such as measurement of pH, foam retention, foam height, determination of percentage of free alkali, alcohol insoluble matter, total fatty matter, moisture content, and saponification, foam forming ability, skin irritation test, antifungal activity were studied

and also a comparative study with herbal bathing soap of medicate clotrimazole soap was performed

CHAPTER- 5

Explain the results and discussion of physiochemical parameters of herbal soap formulation and Comparative study was performed between a branded marketed soap and herbal bathing soap. Brief discussion was done about the study performed.

CHAPTER- 6

Describes the summary conclusion and scope for the future study of the prepared soap.

6.2 CONCLUSIONS

An attempted was made to formulate antifungal herbal soap. The prepared soap showed no irritation on skin. And showed a very good antifungal activity against *Candida albicans* & *Non albican Candida sps* Pathogen. Thus, we can conclude by saying the plant *Macroptilium Lathyroides* and *Acalypha Indica* has a very good antifungal activity.

6.3 FUTURE SCOPE

- To perform *In-Vivo* study for the formulated soap.
- To perform the short term and long-term stability study.
- Establishing the scale up of the prepared soap formulations.

REFERENCES

1. Degreeef, Hugo. "Clinical forms of dermatophytosis (ringworm infection)." *Mycopathologia* 166 (2008): 257-265. DOI: <https://doi.org/10.1007/s11046-008-9101-8>.
2. Jain, Akansha, Shubham Jain, and Swati Rawat " Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention " *Journal of Pharmacy and Bioallied Sciences* 2.4 (2010): 314. DOI: 10.4103/0975-7406.72131.
3. Chen, Sharon CA, and Tania C. Sorrell. " Antifungal agents." *Medical Journal of Australia* 187.7 (2007): 404.
4. Chamilos, Georgios, et al. " Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989- 2003)." *haematologica* 91.7 (2006): 986-989.
5. Schwartz, Robert A. " Superficial fungal infections." *The Lancet* 364.9440 (2004): 1173-1182. DOI: [https://doi.org/10.1016/S0140-6736\(04\)17107-9](https://doi.org/10.1016/S0140-6736(04)17107-9).
6. Ajello, Libero. " Natural history of the dermatophytes and related fungi." *Mycopathologia et Mycologia applicata* 53 (1974): 93-110
DOI: <https://doi.org/10.1007/BF02127200>.
7. Farhan, Rafal K. " Dermatophytes and bacterial super infections in antimicrobial resistant tinea pedis patients in Dour city, Iraq." *European Journal of Molecular & Clinical Medicine* 8.01 (2021): 2021.
8. Davis, John D. " Superficial fungal infections of the skin: Tinea corporis, tinea pedis, and Candida intertrigo." *Primary Care Update for OB/GYNS* 2.5 (1995): 157-161. DOI: [https://doi.org/10.1016/1068-607X\(95\)00033-3](https://doi.org/10.1016/1068-607X(95)00033-3).
9. Ghannoum, M., et al. "Tinea capitis in Cleveland: survey of elementary school students." *Journal of the American Academy of Dermatology* 48.2 (2003): 189-193. DOI:<https://doi.org/10.1067/mjd.2003.109>.

10. Djeridane, Assya, Yasmina Djeridane, and Aomar Ammar-Khodja. & quot; Epidemiological and aetiological study on tinea pedis and onychomycosis in Algeria.& quot; *Mycoses* 49.3 (2006): 190-196

DOI: <https://doi.org/10.1111/j.1439-0507.2006.01230.x>.

11. Kim, Joon, and Peter Sudbery. & quot; *Candida albicans*, a major human fungal pathogen.& quot; *The journal of microbiology* 49 (2011): 171-177

DOI: <https://doi.org/10.1007/s12275-011-1064-7>.

12. Sobel, Jack D. & quot; Genital candidiasis.& quot; *Medicine* 42.7 (2014): 364-368
DOI <https://doi.org/10.1016/j.mpmed.2014.04.006>.

13. Kaushik, Kusum, and S. H. W. E. T. A. Agarwal. & quot; Role of herbal antifungal agents for the management of fungal diseases: a systematic review.& quot; *Asian journal of pharmaceutical and clinical research* 12.7 (2019): 34-40

DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i7.33831>.

14. Hay, Roderick. & quot; Superficial fungal infections.& quot; *Medicine* 41.12 (2013): 716-718. .DOI: <https://doi.org/10.1016/j.mpmed.2013.09.011>

15. Koga, Tetsuya, et al. & quot; Therapeutic approaches to subcutaneous mycoses.& quot; *American journal of clinical dermatology* 4 (2003): 537-543

DOI: <https://doi.org/10.2165/00128071-200304080-00003>

16. Barros, Mônica Bastos de Lima, Rodrigo de Almeida Paes, and Armando Oliveira Schubach. & quot; *Sporothrix schenckii* and Sporotrichosis.& quot; *Clinical microbiology review s* 24.4 (2011): 633-654.

17. Garber, Gary. & quot; An overview of fungal infections.& quot; *Drugs* 61.Suppl 1 (2001): 1-12. 17. DOI: <https://doi.org/10.2165/00003495-200161001-00001>

18. Kirkpatrick, Charles H. & quot; Chronic mucocutaneous candidiasis: immunology for the pediatrician. Edited by Harry R. Hill, MD.& quot; 197-206.

19. Richardson, Malcolm D. " Changing patterns and trends in systemic fungal infections." *Journal of Antimicrobial Chemotherapy* 56.suppl_1 (2005): i5-i11. DOI: <https://doi.org/10.1093/jac/dki218>.
20. Feazel, Leah M., et al. " Opportunistic pathogens enriched in showerhead biofilms." *Proceedings of the national academy of sciences* 106.38 (2009): 16393-16399.
21. Pfaller, M. A., et al. " International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program." *Journal of Clinical Microbiology* 39.9 (2001): 3254-3259. DOI: <https://doi.org/10.1128/JCM.39.9.3254-3259.2001>.
22. Gomerep, S. S., et al. " Frequency of cryptococcal meningitis in HIV-1 infected patients in north central Nigeria." *Nigerian Journal of Medicine* 19.4 (2010): 395-399. DOI: [10.4314/njm.v19i4.61963](https://doi.org/10.4314/njm.v19i4.61963).
23. Gonzalez, Corina E., Michael G. Rinaldi, and Alan M. Sugar. " Zygomycosis." *Infectious Disease Clinics* 16.4 (2002): 895-914
DOI: <https://doi.org/10.1093/clinids/24.5.860>.
24. Yagupsky, Pablo, and Ron Dagan. " *Kingella kingae*: an emerging cause of invasive infections in young children." *Clinical infectious diseases* 24.5 (1997): 860-866.
25. Martinez, Roberto. " New trends in paracoccidioidomycosis epidemiology." *Journal of fungi* 3.1 (2017): 1. DOI: <https://doi.org/10.3390/jof3010001>.
26. Duong, Tuan Anh. " Infection due to *Penicillium marneffe*, an emerging pathogen: review of 155 reported cases." *Clinical infectious diseases* 23.1 (1996): 125-130. DOI: <https://doi.org/10.1093/clinids/23.1.125>.
27. Tripathi, K. D. *Essentials of medical pharmacology*. JP Medical Ltd, (2013).

28. Nirmala, P., and T. Selvaraj. & quot; Anti-inflammatory and anti-bacterial activities of Glycyrrhiza glabra L.& quot; Journal of Agricultural Technology 7.3 (2011): 815-23 ISSN NO: 1686 - 9141.
29. Vila, Roser, Blanca Freixa, and Salvador Cañigueral. & quot; Antifungal compounds from plants." Recent Advances in Pharmaceutical Sciences 3 (2013): 23-43.
30. SS, Jadhav, et al. & quot; Review On Ayurvedic Remedies For Fungal Infection.& quot; ISSN NO: 1007-6735
31. <https://www.myupchar.com/en/disease/fungal-infections/ayurveda>
32. <https://skinlibrary.co.uk/blogs/librarian-edits/skin-barrier>
33. Rao, Mohan. The Stygean Dip History of Cleanliness in France., JSTOR. (1991); 26(34) 1973-1975.
34. Sakkaravarthi, Vinupriya. & quot; History of soap." Cosmoderma 2 (2022).
35. Powar PV, Bhandari NR, Arya Ashwini SP. Formulation and Evaluation of Poly Herbal Anti-Bacterial Gel Based Hand Wash, Int. J. Pharm. Sci. Rev. Res. (2015);33(1):79-82.
36. Devi AS, Sivani DV, Anusha D, Sarath G, Sultana SM. Formulation and Evaluation of Antimicrobial Herbal Soap, Int. J. Pharm. Sci. Rev. Res. (2021); 71(2): 122-125.
37. Pawar, Arti P., Dhanashri N. Pawar, and Yogita V. Dalvi. Formulation and Evaluation of Polyherbal Soap Research Journal of Topical and Cosmetic Sciences (2019); 10(1) 23-28.
38. Kamarajan, G., et al. "Green synthesis of ZnO nanoparticles using *Acalypha indica* leaf extract and their photocatalyst degradation and antibacterial activity." *Journal of the Indian Chemical Society* 99.10 (2022): 100695
- DOI: <https://doi.org/10.1016/j.jics.2022.100695>.

39. Chinnathambi, Arunachalam, *et al.* "Biosynthesis of TiO₂ nanoparticles by *Acalypha indica*; photocatalytic degradation of methylene blue." *Applied Nanoscience* 13, (2021): 1-8, DOI: <https://doi.org/10.1007/s13204-021-01761-3>.
40. Nachiar, G. Shanthini, *et al.*, "Review on antibacterial potency of combination of effective extract of *Tridax procumbens*, *Aegle marmelos*, and *Acalypha indica*." *Journal of Medicinal Plants* 9.5 (2021): 47-50. ISSN NO: 2320-3862.
41. Chekuri, Sudhakar, *et al.*, "*Acalypha indica* L.- an important medicinal plant: a brief review of its pharmacological properties and restorative potential." *European Journal of Medicinal Plants* 31.11 (2020): 1-10. ISSN NO: 2231-0894.
42. Yeng, Ng Kit, *et al.* "Investigation of wound healing effect of *Acalypha indica* extract in sprague dawley rats." *Biomedical and Pharmacology Journal* 12.4 (2019): 1857-1865. **DOI:** <https://dx.doi.org/10.13005/bpj/1816>.
43. Kumar, M. Sathish, *et al.* "*Acalypha indica* and *Curcuma longa* plant extracts mediated ZnS nanoparticles." *Material Science Research India* 16.2 (2019): 174-182. ISSN: 0973-3469 **DOI:** <http://dx.doi.org/10.13005/msri/160210>.
44. Zahidin, Nor Syahiran, *et al.* "A review of *Acalypha indica* L.(*Euphorbiaceae*) as traditional medicinal plant and its therapeutic potential." *Journal of ethnopharmacology* 207 (2017): 146-173
DOI: <https://doi.org/10.1016/j.jep.2017.06.019>.
45. Idris, A.M., *et al.*, "Two newly described begomo viruses of *Macroptilium lathyroides* and common bean." *Phytopathology* 93.7(2003):774-783.
46. Ramos, P.L., *et al.*, "*Macroptilium* yellow mosaic virus, a new begomo virus infecting *Macroptilium lathyroides* in Cuba." *Plant Disease* 86.9(2002):1049-1049.
47. Chauh *et al.*, "Seed germination, seedling emergence, and response to herbicides of wild bush bean (*Macroptilium lathyroides*)." *Wee DOI: 10.1371/journal.pone*.
48. Passos, Laise S., *et al.*, "Complete genome sequence of a new bipartite begomovirus infecting *Macroptilium lathyroides* in Brazil." *Archives of virology* 162 (2017): 3551- 3554.

DOI: <https://doi.org/10.1007/s00705-017-3522-y>

49. J. Bird, *et al.*, "First report of a bean-infecting begomovirus from *Macroptilium lathyroides* in Puerto Rico that is distinct from bean golden mosaic virus." *Plant disease* 83.11 (1999): 1071-1071.

50. Idris AM, Bird J, Brown JK. First Report of a Bean- Infecting Begomovirus from *Macroptilium lathyroides* in Puerto Rico That Is Distinct from Bean Golden Mosaic Virus. *Plant Dis.* (1999) Nov;83(11):1071.

DOI: 10.1094/PDIS.1999.83.11.1071C. PMID: 30841284.

51. Hamad, Mubarak Siddig, *et al.*, "A New Record to the flora of Sudan; *Macroptilium lathyroides* (L.) Urb., Fabaceae." *Journal of The Faculty of Science and Technology* 8 (2021): 49-60.

DOI: <https://doi.org/10.52981/jfst.vi8.1961>.

52. Bracero, V *et al.*, "DNA analysis confirms *Macroptilium lathyroides* as alternative host of Bean golden yellow mosaic virus." *Plant disease* 87.9 (2003): 1022-1025.

53. Lydict, *et al.*, "Detecting bean golden yellow mosaic virus in bean breeding lines and in the common legume weed *Macroptilium lathyroides* in Puerto Rico12."

54. Jithendran, R., and S. Gowri. "*Formulation and Evolution of Polyherbal Soap: A Review Article.*" Volume 9 Issue X Oct (2021) ISSN NO: 2321-9653.

55. Anant, Munde Govind. "Formulation and Evaluation of Herbal Soap by Using Natural Ingredients by Simple Matched." Volume:03/Issue:11/November- (2021) ISSN NO: 2582-5208.

56. Warra, A. A., *et al.* "Antibacterial Activities of Soaps Prepared from Selected Plant Oils." *Int. J. Sci. Res. in Biological Sciences Vol 7.4* (2020). ISSN NO: 2347-7520.

57. Shah, Rutuja R., and Rohan R. Vakhariya. "Formulation and Evaluation of Antifungal Soap of Garlic Oil." *Asian Journal of Pharmaceutical Research* 10.1 (2020): 13-16. ISSN NO: 2231-5683

DOI: <http://dx.doi.org/10.5958/2231-5691.2020.00003.9>.

58. Thirunavukkarasu, Arunachalam, *et al.* "Green soap formulation: an insight into the optimization of preparations and antifungal action." *Biomass Conversion and Biorefinery* (2020): 1-12.

DOI: <https://doi.org/10.1007/s13399-020-01094-1>.

59. Sindhu Rakesh K., *et al.* "Formulation development and antimicrobial evaluation of polyherbal soap." *Plant Arch* 19.2 (2019): 1342-6 ISSN NO: 0972-5210.

60. MV Anoop *et al.*, microbial evaluation of herbal soap containing neem oil and nalpamaradhi oil." *International Journal of chemical and pharmaceutical sciences*" (2015), Dec., Vol. 6 (4) ISSN NO: 0976-9390.

61. <https://www.feedipedia.org/node/627>

62. Priyadharshini, G., R. Praveen Kumar, and N. Praveen Kumar. Formulation and Evaluation of Antifungal Herbal Soap using *Acalypha Indica*. *International Research Journal of Modernization in Engineering Technology and Science* (2022); 04(11): 2256- 2259.

63. Lee, Jeong Ah, and Hee Youn Chee. *In vitro* antifungal activity of equol against *Candida albicans*. *Mycobiology* (2010); 38(4): 328-330.

64. Zhang, Qing-Wen, Li-Gen Lin, and Wen-Cai Ye. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine* (2018);13: 1-26.

65. Reddy, Teja Kumar. Formulate and Evaluate The Herbal Bath Soap using Extracts of Three Plants Having Ethnic and Dermatological Importance in Ayurveda, namely *Azadirachta Indica*, *Curcuma Longa*, *Ocimum Tenuiflorum*. *Neuroquantology* (2022); 20(12): 1055-1062.

66. Kokate C K, Purohit A P, Gokhale S B, Textbook of Pharmacognosy. Nirali Prakashan (2010); 1.4-1.8.
67. Johnson EM, Warnock DW, Luker J, Porter SR, Scully C. Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *J Antimicrob Chemother* (1995); 35: 103-114.
68. Shivaprakasha S, Radhakrishnan K, Karim P. *Candida* spp. other than *Candida albicans*: A major cause of fungaemia in a tertiary care centre. *Indian J Med Microbiol* (2007); 25: 405-407.
69. Akuaden, Namo Jeremiah, I. Y. Chindo, and Joel Ogboji. Formulation, Physicochemical and Antifungi Evaluation of Herbal Soaps of *AzadiractaIndica* and *ZiziphusMauritiana*. *IOSR Journal of Applied Chemistry* (2019); (12) 8: 26-34.
70. <https://www.termpaperwarehouse.com/essay-on/To-Determine-Percentage-Of-Free-Alkali/167256>.
71. Vasanthan.A *et al.*, "Formulation and evaluation of antifungal herbal soap using *Acalypha Indica*" *International Research journal of modernization in engineering technology and science*. 04, (2022), 2256-2259, ISSN NO: 2582-5208.