

**FORMULATION AND EVALUATION OF VARIOUS ANTI-
MICROBIAL COSMETIC PRODUCTS USING
Sphagneticola trilobata (L.) Pruski**

Submitted in partial fulfillment of the requirements for the award of

BACHELOR OF PHARMACY

BY

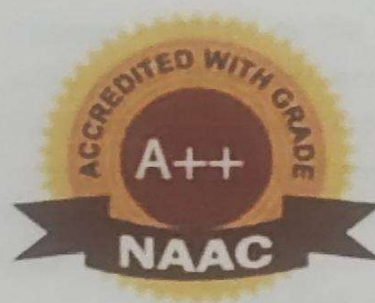
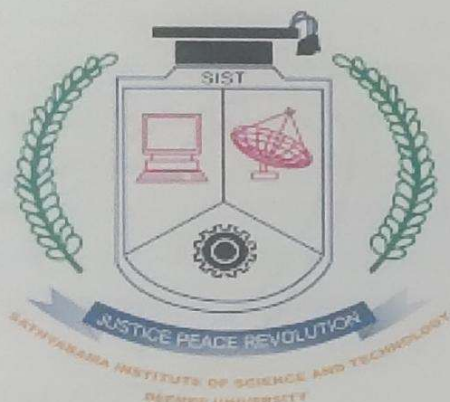
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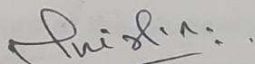
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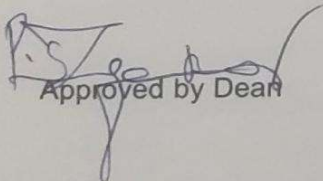
BONAFIDE CERTIFICATE

This is to certify that the thesis entitled "**FORMULATION AND EVALUATION OF VARIOUS ANTI-MICROBIAL COSMETIC PRODUCTS USING *Sphagneticola trilobata* (L.) Pruski**" submitted by "**ANISHIYA M (40050006) KESAVARDHINI D (40050010) KAMESH K (40050021) SUJIPRIYA R (40050052) HARIHARA SUTHAN S (40050702)**" to the Sathyabama Institute of Science and Technology (Deemed to be University), Chennai - 600119, for the award of **Bachelor of Pharmacy** by research is a bonafide record of research work carried out by them under my supervision. The contents of this thesis, in full or in part, have not been submitted to any other institute or university for the award of any degree, diploma, associate-ship, fellowship or any similar title and that it represents entirely an independent work on the part of the candidate.

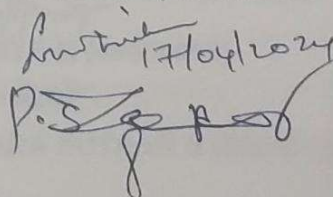
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DECLARATION

We hereby declare that the work presented in the thesis entitled **“FORMULATION AND EVALUATION OF VARIOUS ANTI-MICROBIAL COSMETIC PRODUCTS USING *Sphagneticola trilobata* (L.) Pruski”** submitted by us to the **Sathyabama Institute of Science and Technology (Deemed to be University), Chennai - 600119**, for the award of **Bachelor of Pharmacy (2020-2024)** by research is an original record of research work carried out by us under the supervision of **“Mrs.S.Monisha**, 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, diploma, membership, fellowship, associate-ship etc... In keeping with the general practicing 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

OBJECTIVE: This study is to investigate the antimicrobial properties of *S.trilobata* and to formulate and evaluate various antimicrobial cosmetics utilizing different parts of the plant.

METHODS: The antimicrobial activity of *S.trilobata* is assessed through experimentation, targeting both Gram-positive and Gram-negative bacteria, as well as yeasts. Various extracts of plant are tested against a panel of microbial strains to ascertain their efficacy. Anti-fungal herbal formulations including shampoos, soaps, and handwashes are prepared and evaluated.

RESULTS: The plant extracts exhibit inhibitory effects on Gram-positive bacteria - *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and yeast *Candida albicans*. Herbal formulations developed using *S.trilobata* exhibit promising anti-fungal efficacy, suggesting their potential application in cosmetic products.

CONCLUSION: The findings highlight the feasibility of utilizing *S.trilobata* in pharmaceutical formulations and cosmetics due to its broad-spectrum antimicrobial activity. Moreover, the study emphasizes to explore natural alternatives to synthetic, aligning with the growing demand for safer and more sustainable cosmetic products.

Keywords: *Sphagneticola trilobata*, Phytochemical screening, Anti-microbial activity, Herbal Formulations, Evaluations.

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<i>S. trilobata</i>	<i>Sphagneticol trilobata</i>
<i>W. trilobata</i>	<i>Wedelia trilobata</i>
(L.)	Linn
<i>E. Coli</i>	<i>Escherichia coli</i>
<i>S. Aureus</i>	<i>Staphylococcus aureus</i>
<i>C. Albicans</i>	<i>Candida albicans</i>
<i>P. Vulgaris</i>	<i>Proteus vulgaris</i>
<i>B. thuringensis</i>	<i>Bacillus thuringensis</i>
MRSA	Methicillin-Resistant <i>Staphylococcus Aureus</i>
Iv	Intravenous
Uti	Urinary Tract Infection
Expec	Extraintestinal Pathogenic <i>Escherichia Coli</i>
IgA	Immunoglobulin A
IgG	Immunoglobulin G
TSST-1	Toxic Shock Syndrome Toxin-1
PVL	Panton-Valentine Leukocidin
IPEC	Intestinal Pathogenic <i>E. Coli</i>
<i>Z. mauritiana</i>	<i>Ziziphus mauritiana</i>
HPMC	Hydroxypropylmethylcellulose

Zr-NPs	Zirconiumnanoparticles
Ag-NPs	Silvernanoparticles
AgNO ₃	Silvernitrate
KLO	KaffirLimeOil
KOO	KingOrangeOil
KLJ	KaffirLime Juice
LGO	LemonGrassOil
RNE	Rinacanthusnasutusextract
MTE	Mangosteenextract
SLS	Sodiumlaurylsulphate
g	Gram
mL	Milliliter
μL	Microliter
mm	Millimeter
cm	Centimeter
cP	Centipoise
CC	CarrierSolventControl
PC	Positive Control
MHA	MullerHintonAgar
ZOI	Zoneofinhibition

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CHAPTER -1

INTRODUCTION

CHAPTER-1 INTRODUCTION

Table1.1: Taxonomical Classification of *Sphagneticolatrlobata*¹

Biological name	<i>Sphagneticolatrlobata</i>
TAXONOMICAL CLASSIFICATION	
Kingdom	Plantae
Tibe	Heliantheae
Family	Asteraceae
Order	Asteraceae
Genus	<i>Sphagneticola</i>
Species	<i>Sphagneticolatrlobata</i>
Tamil Name	மஞ்சள்கரம்பு /பாலி கரிசலாங்கண் ணி
Synonyms	<i>Camplalya trilobata</i> , <i>Silhium trilobata</i> , <i>Wedeliatrlobata</i> , <i>Serunenum trilobatum</i> , <i>Telechitonia trilobata</i> , <i>Stemmodontia</i>



Figure1.1 The whole plant of *Sphagneticolatrlobata*

CULTIVATION:

The plant *S. trilobata* grows based on the stem and stem fragments spread over the ground mostly in the garden waste. When they are exposed to the ground they develop into a new plant and mostly during the floods, they are grown in larger quantities. Viable seeds are said to be rare; however, 17% viability has been reported from Fiji². It is widely cultivated as an ornamental groundcover.

S. trilobata is a very important invasive weed; a perennial creeping plant forming extensive, dense ground cover, crowding out other species. A weed of urban bushland, closed forests, forest margins, open woodlands, waterways, lake margins, wetlands, roadsides, disturbed sites, waste areas, vacant lots, and coastal sand dunes in tropical and sub-tropical regions. It may also grow into lawns, footpaths and parks from nearby gardens, mostly they prefer well-drained, moist soil, and sunny areas, but also reported to be equally suited to dry sites. In the Pacific islands, it has become invasive along streams, canals, the margins of mangrove forests and in coastal vegetation³

GEOGRAPHICAL SOURCE:

The geographical source of *S. trilobata* is found to be the Caribbean, but now grows throughout the Neotropics⁴. The plants are distributed widely among Africa, Asia, North, South and Central America, the Caribbean, Europe, Oceania. It is recorded from Australia, American Samoa, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Nauru, New Caledonia, Niue, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, Tokelau, Tonga and Vanuatu. *S. trilobata* is native to Mexico, Central America, the Caribbean and tropical South America.⁵

THERAPEUTIC USE:

S. trilobata (L.) is a medicinal plant known for its therapeutic effects for ulcer, sore throat, varicose, headache, fever, epilepsy, amenorrhea, snakebite, wounds, kidney dysfunction, hepatitis, cold, and indigestion.⁶ Some literatures have reported the plant's bioactivities such as antioxidant, antibacterial, anti-inflammes, and antimalarial, antifungals, hepatoprotective, antidiabetic, and antitumor.^{7,8,9} The plant has traditionally been used to treat infections, indigestion and hepatitis. It shows

considerable anti-hepatotoxic and protective effects against liver damage. The active ingredient present in the plant is a coumestan called wedelolactone.¹⁰

Sphagneticola trilobata's leaves or aerial parts are used for backache, musclecramp,rheumatism,sores,swellingandarthriticpainful joints¹¹.Theleaves and stems are used in childbirth and in the treatment of bites and stings, fever and infection¹², wound healing¹¹. *S.trilobata* has antioxidant¹³, anti-bacterial¹⁴, analgesic¹⁵ and antimicrobial activities¹⁶. It is a potential candidate in the management of diabetes¹⁷.

The pharmacological activities may depend on its phytoconstituents, the secondary metabolites such as tannin, saponins, flavonoids, phenol and terpenoids.¹²It was reported that numerous potential bioactive molecules such as sesquiterpenes, diterpenes, (Kaurenoic acid), triterpenes lactones, luteolin and volatile oil, with antioxidant, anti-inflammatory, antimicrobial, hepato-protective activity, insecticidal, larvicidal and trypanocide activity, anticancer , anti-tumoral activity have been isolated fromvarious parts of the plant*S.trilobata*.^{15,18} due to its Secondary metabolites.

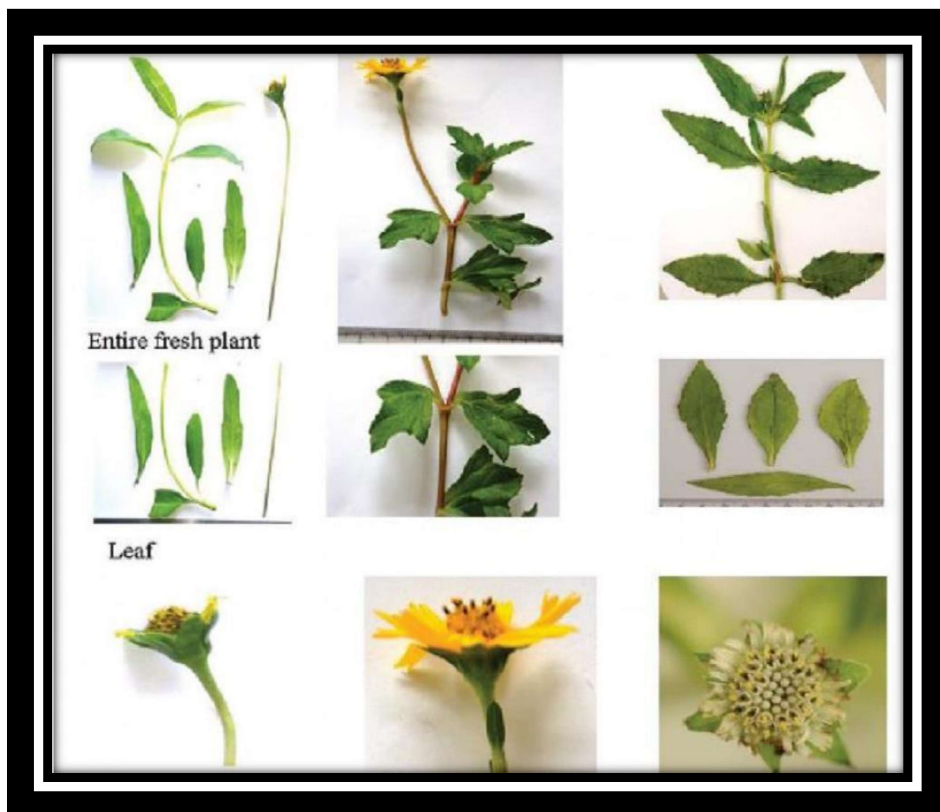


Figure1.2Variouspartsof*Sphagneticolatrilobata*¹⁹

ANTI-MICROBIALACTIVITY:

The Biological screening against Gram-positive Gram-negative bacteria, and fungi of crude extracts from *S.trilobata* has anti-microbial activity, due to the presence of chemical constituent - n-hexane. The biological screening has anti-bacterial against *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium smegmatis*, and *Staphylococcus epidermidis* (Gram-positive bacteria); and against *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, and *Shigella sonnei* (Gram-negative bacteria). The Extracts also showed biological activity against the yeasts (*Candida albicans*, *Candida tropicalis*, *Rhodotorula rubra*) or the fungi (*Aspergillus flavus*, *Aspergillus niger*, *Mucor* sp., *Trichophyton rubrum*)¹⁵ The plant extract of Ethanol, Methanol, Ethyl Acetate, Petroleum Ether etc., showed antibacterial activity against tested bacterial pathogens (*Bacillus thuringensis*, *Bacillus megaterium*, *Streptococcus lactis*, *E. coli*, *Proteus vulgaris*) except aqueous extract.

Among the above mentioned extracts, ethyl acetate extract of leaves shown maximum activity against all tested pathogens with a maximum zone of inhibition in *E. coli* 19.5 mm, *S. lactis* and *P. vulgaris* 18 mm zone of inhibition. Ethyl acetate extract of the stem shown significant antimicrobial activity against all tested bacterial pathogens with a zone of inhibition in *B. thuringensis* 16 mm, *S. lactis* 17.5 mm zone of inhibition. The whole plant of ethyl acetate extract exhibited maximum antimicrobial activity against *E. coli* 16 mm, *S. lactis* 15.5 mm zone of inhibition.¹⁵ Comparative analysis of the antimicrobial activity of different parts of *S.trilobata* revealed that ethyl acetate extract of leaves shown an inhibitory effect against most tested bacterial pathogens.

BENEFITS OF HERBAL PRODUCTS:

The spectacular rise in the utilization of the herbal plants as well as its extracts gave rise to a newer branch of medicinal science referred as 'Herbal Medicinal Products'. These may be defined as the plant or part of plant used as a whole or as an extract for the treatment, prevention of disease or ailment to be utilized in the health care management.²⁰ The herbal medicine imparting various properties are used in pharmaceutical formulations for the treatment of various skin related disorders²¹ as well as for the cosmetic use.²²

Herbal plants with medicinal properties are being used as a traditional medicine from times immemorial. The extract from the leaves, stem and roots of various medicinal plants have been employed as a natural remedy in curing various ailments and diseases. Even though many plant based products have been replaced by synthetic chemicals, the safety and efficacy of ayurvedic products could not find their match²³. The active constituents responsible for such medicinal values are isolated and employed topically as creams, soaps, oils and ointments for treating skin related ailments like acne²⁴, wounds²⁵, eczemas, ring-worms, as an anti-microbial agent²⁴ and for cosmetic purposes.

MICROBIALS:

Species *Staphylococcus aureus* (*S. aureus*)

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that has a spherical shape and is a member of the Bacillota. It is a common member of the human microbiota and is often found on the skin and in the upper respiratory tract. *S. aureus* can be a commensal, but it can also become an opportunistic pathogen that causes skin infections, respiratory infections, and food poisoning.

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations²⁶. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*).^{26,28} *S. aureus* is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals.²⁸ *S. aureus* does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections.²⁸ Transmission is typically from direct contact. However, some infections involve other transmission methods.²⁹

Staphylococcus aureus (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major reservoir for these organisms.^{30,31} It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carry *S. aureus* in the anterior nares. Some populations tend to have higher rates of *S. aureus* colonization (up to 80%), such as healthcare workers, persons who use needles on a regular basis

(i.e., diabetics and intravenous (IV) drug users), hospitalized patients, and immunocompromised individuals. *S. aureus* can be transmitted person-to-person by direct contact or by fomites.^{32,30,27}

S. aureus are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections.³³ Depending on the strains involved and the site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases.^{33,34} The pathophysiology varies greatly depending on the type of *S. aureus* infection.³³ Mechanisms for evasion of the host immune response include the production of an antiphagocytic capsule, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and blocking chemotaxis of leukocytes.^{32,34} Binding of the bacteria to extracellular matrix proteins and fibronectin in infectious endocarditis is mediated by bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids.³⁶ Also, Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) are important virulence factors in infectious endocarditis, sepsis, as well as toxic shock syndrome.^{34,45} Pneumonia infections are associated with the bacterial production of PVL (Panton-Valentine leukocidin) Prosthetic device infections are often mediated by the ability of *S. aureus* strains to form biofilms as well as communicate using quorum sensing in a bacterial cell density-dependent manner.³⁶

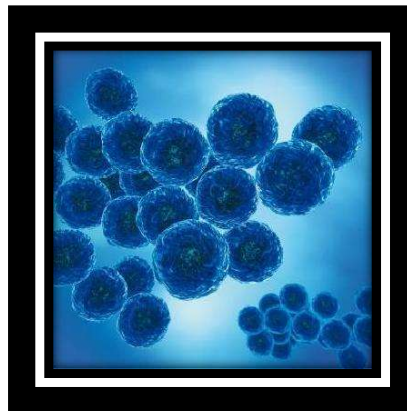


Figure 1.3 *Staphylococcus aureus* (*S. aureus*)²⁶

Species *Escherichia coli*

The bacteria *Escherichia coli* was discovered by German pediatrician Theodor Escherich (1857-1911), who isolated it from babies' feces in 1885^[37]. *E. coli* is a gram-negative, non-sporulating, rod-shaped, facultative anaerobic, and coliform bacterium pertaining to the genus *Escherichia* that commonly inhabits the environment, foods, and warm-blooded animals' lower gut³⁸. In the domains of biotechnology and microbiology, it is the most widely studied prokaryotic model organism. It can live for long periods of time in feces, soil, and water, and is frequently used as a water contamination indicator organism. For 2-3 days, the bacterium multiplies rapidly in fresh feces under aerobic circumstances, but its numbers gradually fall after that. *E. coli* is gram-negative, straight, rod-shaped, non-sporing, non-acid fast, and bacilli that exist in single and pairs. Cells are typically rod-shaped, with $1-3\mu\text{m} \times 0.4-0.7\mu\text{m}$ (micrometer) in size around $1\mu\text{m}$ long, $0.35\mu\text{m}$ wide, and $0.6-0.7\mu\text{m}$ in volume³⁹. It is motile due to peritrichous flagellar arrangement, and very few strains are non-motile. The optimal growth of *E. coli* occurs at 37°C (98°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). It takes as little as 20 min to reproduce in favorable conditions⁴⁰. Fimbriated strains exist both as motile and non-motile. A polysaccharide capsule has been discovered in some *E. coli* strains isolated from extraintestinal infections. The *E. coli* capsules can be clearly seen using negative staining procedures, which produce a bright halo over a dark backdrop. They have a thin cell wall with only one or two layers of peptidoglycan.⁴¹

The majority of *E. coli* strains in the colon are not harmful, however pathogenic *E. coli* isolates cause intestinal or extraintestinal infections, depending on the array of virulence-associated genes that they harbor. The intestinal pathogenic *E. coli* (IPEC) strains are divided and classified into several pathotypes. Diseases associated with various intestinal pathogenic *E. coli* pathotypes in animals. *E. coli* is linked also to a number of extraintestinal diseases and is the most prevalent cause of cholecystitis, bacteremia, cholangitis, UTI, traveler's diarrhea, and septicemia as well as neonatal meningitis, etc.,⁴². Most infections, with the exception of infant meningitis and gastroenteritis, are endogenous, like *E. coli* from the patient's normal microbiota, cause infection when the patient's defenses are impaired, e.g., through trauma or immune suppression⁴³. In order to cause disease *E. coli* possesses several different types of virulence factors: fimbrial and

fimbrial adhesins, capsules, toxins (exotoxins, hemolysins, and enterotoxins), iron up-take systems, etc.,⁴⁴. Important ExPEC virulence-associated genes, their encoded proteins, function, and connection to different ExPEC pathotypes.



Figure 1.4 *Escherichia coli*³⁸

Species *Candida albicans*:

Candida albicans is a fungus that naturally lives on your body. *Candida* is yeast, a type of fungus, that is typically found in small amounts on your mouth, skin and in your intestines. Healthy bacteria in your body (microbiome) control the balance of *Candida*. Often when it is off-balance, the yeast overgrows and causes infection (candidiasis).

Yeast infections are common because the balance of *Candida* in your body can easily become disrupted. The most common infection caused by *albicans* is a vaginal yeast infection, which occurs in about 75% of people with a vagina at least once in their lifetime.

C. albicans carriage is asymptomatic in most individuals and disease typically arises when perturbation of host homeostasis and/or endogenous microbiota occurs. It was recently shown that administration of β -lactam antibiotics causes the release of bacterial peptidoglycan subunits—including tracheal cytotoxin among others—that induces the invasive hyphal fungal program in the gut.⁴⁵ Such antibiotic-induced perturbations, immune system abnormalities (see below), changes in the microbiome, and/or changes in mucocutaneous barrier integrity^{46,47} enable *C. albicans* to become an opportunistic pathogen in the context of expression of an array of virulence determinants.

Recently, it was shown that immune selection by intestinal IgA against *C. albicans* filaments suppresses harmful fungal effectors while improving the competitive fitness of *C. albicans* yeast as a commensal⁴⁸. Within the gut, *C. albicans* helps shape the composition of the healthy microbiota by inhibiting multiple dominant genera of gut bacteria⁴⁹ and local inflammation⁵⁰. The presence of *C. albicans* in the gut is linked to an increase in splenic IgG-producing B cells and systemic antifungal IgG conferring protection against candidemia^{49,51,52}. In mice, multiple passages of *C. albicans* resulted in fungal adaptation toward colonization and improved protection against subsequent nonspecific infections in a lymphocyte-independent manner.⁵³

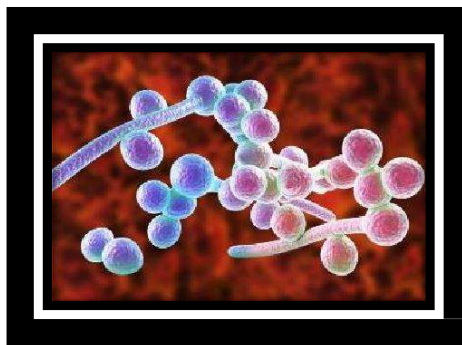


Figure 1.5 *Candida albicans*⁴⁵

FORMULATION OF HERBAL COSMETICS:

Anti-Microbial Soap:

Soaps are used for cosmetic use. Soaps are defined as the cleaning agents—solid, liquid, semisolid powders which help in removing dust, dirt, microorganisms, stains and for maintaining health and beauty.²⁸ Chemically soaps are the combination of fats, oils (of animal or vegetable origin) and Salt.²⁴ Soaps are generally salts of free fatty acid made via saponification. Fatty acids such as lauric acid, palmitic acid, stearic acid help in imparting washing property of soaps.⁵⁴ where alkaline substances react with fatty acids in fats or oils. Other substances are then added to this salt of free fatty acid or glycerine base, to produce the different types of soaps we have. They are mainly used as surfactants for washing, bathing and cleaning.⁵⁴ Soaps are either nonantimicrobial soaps or antimicrobial soap, also known as an antiseptic or medicated soap.

An antibacterial soap can remove 65% to 85% of bacteria from human skin.³⁹ Antiseptic soaps are incorporated with specified amount of germicidal substances in addition to the ordinary soap base in order to increase their antibacterial activity. These antiseptic substances impart ability for the soap to kill germs even after it has been used as residual antiseptic substances remain on the skin. It is proved experimentally that antibacterial soaps kill the bacteria at a specific concentration; they also have bacteriostatic activity and can inhibit the growth of bacteria.

Herbal soaps are prepared by adding dried herbs, flowers and stems into soap base. Herbs are the natural products could be found in the treatment of almost all diseases and skin problems owing to their high medicinal value, cost-effectiveness, availability and compatibility.^{55,56} Hence it can be used in soap base. The attribute of the soap includes gentleness on the skin, rich lather, protection against skin disorders (including rashes, eczema, scabies) treatment of skin infection (such as ringworm), protection of even skin toning and smoothness of the skin.⁵⁷

The most alluring quality of the handmade soap is their harmless effect on your skin. The handmade soaps improve your skin condition. It relieves from itchiness, dryness, and skin conditions like eczema, and acne. The chemical soap removes natural skin oil, which protects and lubricates the skin. These soaps also might lead to breakouts or dry skin. These types of soaps will not affect you in a day or month usage but it will have its effect over years of usage.

Anti-Microbial Shampoo:

Shampoos are probably the most widely used cosmetic products for cleansing hairs and scalp in our daily life.⁵⁸ A shampoo is basically a solution of a detergent containing suitable additives for other benefits such as hair-conditioning enhancement, lubrication, medication etc. Now-a-days many synthetic, herbal, medicated and non-medicated shampoos are available in the market but popularity of herbal shampoo among consumers is on rise because of their belief that these products being of natural origin are safe and free from side effects.⁵⁹ Synthetic surfactants are added to shampoo primarily for the foaming and cleansing action but their regular use leads to dryness of hairs, hair loss, irritation to scalp and eyes.⁶⁰

Herbal formulations are considered as alternative to synthetic shampoo but formulating cosmetics using completely natural raw material is a difficult task.⁶¹ There are large numbers of medicinal plants which are reported to have beneficial effects on hair and are commonly used in formulation of shampoo.⁶² These plant products may be used in their powdered form, crude form, purified extracts, or derivative form.⁶³ It is extremely difficult to prepare a herbal shampoo using a single natural material that would be milder and safer than the synthetic ones, and at the same time would compete favorably with its foaming, detergency and solid content. We, therefore, considered to formulate an herbal shampoo using traditionally and commonly used plant materials for hair washing.⁶⁴

Chemical Shampoos contain sulfate, which is responsible for creating foam and lather. Sulfate is a harsh chemical that takes away your hair's natural oil and destroys its pH level. On the contrary, natural shampoos are sulfate free, which eliminates the dirt from your hair that collects on your hair follicle without disturbing pH levels and natural oil balance. Additionally, regular shampoos contain harmful chemicals such as parabens, silicones, dyes, artificial fragrances, etc. Natural shampoos contain ingredients derived straight from nature.⁶⁵ These ingredients combine to form a solution that works on the root cause of hair problems and improves its quality.

Anti-Microbial Handwash:

Hands are the primary area for transmission of germs and infection. Hand hygiene is thus the most important measure to avoid the spread of harmful germs and the spread of healthcare associated infections. Hand washing is the act of cleaning one's hand to remove dirt and pathogenic microorganisms and to prevent the transmission of transient microorganisms.⁶⁶ Plants, on the other hand, have complicated compositions and their therapeutic activities are dependent on their major active chemical constituents. In addition, improper herb authentication, microorganism adulteration, and pesticide residue have made herbal drug standardization critical. As a result, before using these herbs in any formulation, their confirmation is required. Traditional medicine has been used for a longer period of time to prevent infectious diseases.⁶⁷ Given this deadline, an attempt was made to screen classical literature for herbs with antimicrobial properties and it was

discovered that *S.trilobata* extract possess that antimicrobial potency. As a result, we set out to make and test an herbal handwash made up of alcoholic extracts.

Hand washing removes visible dirt from hands and reduce the number of harmful microorganismssuchas,*E.Coli*and*S.aureus*.⁶⁸TheHerbalHandwashare veryeffectiveagainstvariousbacteria,virusesandother microbeswhen compared to chemical handwash.⁶⁹ Herbal Handwash is very effective, environment friendly, biodegradable and inexpensive. Herbal Handwash is much better than the plain soap due to their ingredients and effectiveness on our skin of hands as well as suitable for all type of skin. Mostly herbal Handwash protect us from many daily encountering bacteria's.⁷⁰

CHAPTER -2
LITERATURESURVEY

CHAPTER-2

REVIEW OF LITERATURE

LITERATURE REVIEW ON ANTIMICROBIAL ACTIVITY OF *Sphagneticola trilobata* EXTRACTS

Dinusha M. Jayarathne et al., (2024) conducted a study on “Identifying New Pathogen for Black Rot Disease of Tea and Incorporated *Wedelia trilobata* Extraction and Captan for Controlling Black Rot Disease.” According to this study, *Daldinia eschscholtzii* can spread black rot disease as a causal fungus; *Corticium theae* and *Corticium invisum*. Ethyl acetate extract of stem, flower and leaves of *Wedelia trilobata* and Captan had antimicrobial activity against the *Daldinia eschscholtzii*. Stem extract was the effective extract for inhibiting the growth of the black rot pathogen.⁷¹

Wen-xing Li et al., (2022) conducted a study on “Two new lactam derivatives from a *Sphagneticola trilobata* derived fungus *Penicillium rubens* PQQ-2.” This study concludes that a new bicyclic lactam derivative penicilactam B and a new monocyclic amide penicillamide D, along with four known compounds were evaluated for their antibacterial activities. They were found to be active against *Staphylococcus aureus* subspecies, *Candida albicans*, *Escherichia coli*.⁷²

Remya et al., (2021) conducted a study on “Comparative Evaluation of Phytochemical and Antibacterial Activity of Different Parts of *Sphagneticola trilobata* (L.) Pruski.” This study concludes that the methanolic extract of the stem, leaves and whole plant exhibited moderate antimicrobial activity. Petroleum ether extracts showed the least antimicrobial activity. Aqueous extracts of plant parts have no inhibitory action against all tested pathogens. Among them ethyl acetate extract of leaves showed maximum activity against *Escherichia coli*, *Streptococcus lactis* and *Pemphigus vulgaris*. Ethyl acetate of the stem also showed antimicrobial activity against *Streptococcus lactis* and *Bacillus thuringiensis*. Whole plant ethyl acetate extract exhibited maximum antimicrobial activity against *Escherichia coli* & *Streptococcus lactis*. Thus, it indicates the possibility of using leaves, stem and whole plant of *Sphagneticola trilobata* as natural antimicrobial agents.⁷³

Shi-Fei Li et al., (2016) proposed a study on "Antimicrobial Diterpenoids of *Wedelia trilobata* (L.) Hitchc." This study concludes that diterpenes and sesquiterpenes are the main metabolites of *Wedelia trilobata* and they may be significant for antimicrobial activities against a panel of bacteria and fungi. They show strong activity against the following tested organisms *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Monilia albicans*, *Escherichia coli* and showed weak activities against *Monilia albicans*.⁷⁴

Merell Pabilona Billacura et al., (2015) conducted a study on "Leaf Extract Mediated Green Synthesis of Silver Nanoparticles from widely available *Wedelia Trilobata*: Synthesis, Partial Characterization and Antimicrobial Property analysis." This study concludes that the silver nanoparticles were synthesized by reacting silver nitrate with the plant extract were evaluated for antimicrobial activity. The results show that the water extract of *Wedelia trilobata* alone does not inhibit *Escherichia coli* and *Staphylococcus aureus* growth and the silver nitrate solution showed slight bacterial growth inhibition but the addition of the plant extract to silver nitrate increases the zone of inhibition.⁷⁵

Ram S. Verma et al., (2014) performed a study on "Essential oil composition of *Sphagneticola trilobata* (L.) Pruski from India." This study concludes that the major constituents of the volatile oils were α -pinene (78.6-83.3%), α -phellandrene (1.3-4.1%), sabinene (1.4-1.9%), limonene (1.2-1.9%), β -pinene (1.0-1.6%), camphene (0.7-2.0%), 10-nor-calamenen-10-one (<0.05-1.5%), germacrene D (0.1-1.4%) and γ -amorphene (<0.05-1.3%). These volatile oils are the reasons for showing the antimicrobial activity.⁷⁶

Sureshkumar et al., (2007) conducted a study on "Antimicrobiological Studies on Different Essential Oils of *Wedelia* Species (*W. chinensis*, *W. trilobata* and *W. biflora*) and *Eclipta alba* (Asteraceae)." This study concludes that the essential oil of *Wedelia trilobata* was found to be effective against gram-positive and active against all tested fungi. While the gram-negative organisms show a moderate activity (= 50 %) at the tested concentrations compared to standards. The fungal activities of the oils may be due to the presence of terpenoids, steroids and flavonoids.⁷⁷

S.A.Nirmaletal.,(2005)conductedastudyon“Chemical compositionand antimicrobial activity of essential oil of *Wedelia trilobata* leaves.” This study concludes that the essential oil obtained from *Wedelia trilobata* leaves contains fourteen constituents of which α -pinene (78.64%), D-limonene (2.97%) and germacrene D (3.91%) were the dominant compounds. The antibacterial activityof the essential oil was evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.⁷⁸

TaddeiA.etal.,(1999)performedastudyon“Antimicrobialactivity of*Wedeliatrilobata*crudeextracts”.Thisstudy concludesthatthe n-hexaneextract showed antibacterial activity against *Bacillus subtilis*, *Mycobacterium smegmatis*, *Staphylococcus aureus* and*Staphylococcus epidermidis* (Gram-positive bacteria); along with *Proteus vulgaris*, *Pseudomonas aeruginosa*, *SalmonellagroupC*, *Salmonellaparatyphi*and *Shigellasonnei*(Gram-negative bacteria). The ethyl acetate extract was active against *SalmonellagroupC*and the aqueous extract was inactive against the tested bacteria.¹⁵

LITERATUREREVIEWONANTIBACTERIALACTIVITYOF *Sphagneticola trilobata* EXTRACTS

B.Sowmyaetal.,(2023)conductedastudyon“Employingnewlydeveloped copper oxide nanoparticles for antibacterial capability from discarded *Wedelia trilobata* flowers.” This study concludes that the copper oxide nanoparticles were synthesized utilizing an aqueous petal extract of*Wedelia trilobata* as a reducing agent. Antibacterial activity of nanoparticles is measured by minimal inhibitory concentrationassayfor whichthree differentbacterial strainsareused *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.⁷⁹

SangeetaSSutareetal.,(2020)proposeda studyon“Anatomical studiesof *Sphagneticola trilobata* (L.) Pruski. (Asteraceae).” This study concludes that the phytochemical studies reveal that starch, proteins and alkaloids are present in the root; tannins, saponins and fats are present in stem and leaves, absent in the root and glycosides are present in the leaf and absent in the stem and root. Due to the presence of these active chemicals ethyl extract of*Sphagneticola trilobata* shows

antibacterial activity.⁸⁰

Nuttakorn Baisaeng et al., (2017) conducted a study on “Antibacterial activity of essential oil from *Wedelia trilobata* leaves against *Propionibacterium granulosum*.” This study concludes that the hydro-distilled essential oil of *Wedelia trilobata* leaves possesses antibacterial activity against *Propionibacterium granulosum* compared to commercial clindamycin and benzoyl peroxide gels. This may be due to the synergistic effects of α -pinene and other bioactive compounds such as monoterpenes, α -phellandrene (7.4%), limonene (3.6%), oxazine (3.3%), and β -pinene (3.1%) present in the oil.⁸¹

Chan WeiYang(2015) performed a study on “Bioassay-Guided Isolation of Bio-Active Antibacterial compounds from the Leaves of *Wedelia trilobata*.” This study concludes that the inhibition zones exhibited by various solvent extracts of *Wedelia trilobata* against the bacterial strains of *Staphylococcus epidermidis* and *Staphylococcus aureus* respectively possess antibacterial activity.⁸²

LITERATURE REVIEW ON ANTIFUNGAL ACTIVITY OF *Sphagneticola trilobata* EXTRACTS

Sharif Kaziet al., (2022) performed a study on “Green Synthesis, Characterization and Bio-evaluation of Zirconium Nanoparticles Using the Dried Biomass of *Sphagneticola trilobata* Plant Leaf.” This study concludes that the green synthesis of zirconium nanoparticles (Zr-NPs) was carried out via the reduction of aqueous zirconyl nitrate using leaf extract of *Sphagneticola trilobata* under ultrasound irradiations. They are greener, efficient and cost-effective approaches. The electron diffraction pattern reflects its crystalline nature. Synthesized Zr-NPs showed antifungal activity against *Candida albicans* and *Aspergillus niger*.⁸³

Anne Masih(2022) proposed a study on “Antimicrobial and Catalytic Activity of Biosynthesized Silver Nanoparticles.” This study concludes that the production of silver nanoparticles (Ag-NPs) using *Crinum Asiaticum* and *Sphagneticola trilobata* plants produces anti-microbial properties and is also used for fungal

treatment, reducing toxic dyes efficiently and is inexpensive. The antifungal assay

was carried out using *Candida* isolates which cause life-threatening fungal infections and finding their respective zone of inhibition treated with Ag-NPs, AgNO₃ and using fluconazole as a control.⁸⁴

LITERATURE REVIEW ON PHYTOCHEMICAL SCREENING OF *Sphagneticola trilobata* EXTRACTS

Sushmita Langhi et al., (2020) conducted a study on “Pharmacognostic and phytochemical investigation of *Wedelia trilobata* leaves.” This study concludes that the preliminary phytochemical screening mainly reveals the presence of steroids and triterpenes in petroleum ether extract; alkaloids in chloroform extract; flavonoids in ethyl acetate extracts; carbohydrate, reducing sugars, tannin and glycoside in methanol extract.⁸⁵

Sushama Raj RV (2019) conducted a study on “Primary phytochemical investigation of *Sphagneticola trilobata* (L.) Puruski”. This study concludes that the phytochemical screening of the ethanol, petroleum ether, chloroform and distilled water extracts from powdered leaves, stems and roots of *Sphagneticola trilobata* reveals the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, proteins, amino acids and carbohydrates in all extracts. More phytochemicals were found present in the extract prepared with ethanol. Quinones, phlobatannins and oxalates were absent in all extracts from different parts of the plant.⁸⁶

C. Karthika et al., (2018) conducted a study on “Pharmacognostic, physicochemical analysis and phytochemical screening of the leaves of *W. trilobata* L.” This study concludes that leaves contain a number of pharmaceutically important phytochemicals like Alkaloids, Saponins, Flavonoids, Terpenoids, Tannins, Anthraquinones, Carbohydrates and Protein which could be used as a diagnostic tool for the standardization of this medicinal plant and will be helpful in the characterization of the crude drug.⁸⁷

Neelam Balekare et al., (2014) conducted a study on “*Wedelia trilobata* L.: A Phytochemical and Pharmacological Review.” This study concludes that *Wedelia*

trilobata flowers, leaves and stems were extracted with N-hexane, ethyl acetate, N-butyl alcohol and water to evaluate their antimicrobial activity. Among those solvents, the ethyl acetate was the most effective as it shows the most potent antimicrobial activities.¹³

LITERATURE REVIEW ON FORMULATION OF HERBAL SOAP

Jitendra Pandey et al., (2023) conducted a study on “Exploring the potentials of *Ziziphus mauritiana* Lam. seed kernel oil as pharmaceutical oil base: Physicochemical characterization and ketoconazole soap formulation.” This study explains the formulation of antifungal soap using ketoconazole with *Ziziphus mauritiana* seed kernel oil as a natural base. The formulated antifungal soaps with natural oil may provide several additional benefits, such as moisturization, skin soothing effects, hair and skin rejuvenation, and the absence of carcinogenic risk associated with synthetic soaps.⁸⁸

Nirupama Wijayawardhana et al., (2021) conducted a study on “Antimicrobial activity of a combination of three natural plant extracts and development of a herbal soap.” This study is aimed to evaluate the antimicrobial activity of *Azadirachta indica*, *Cassia fistula*, *Nelumbo nucifera* and their combined extract to formulate an antimicrobial herbal soap. The physical and chemical parameters of the formulated herbal soap were evaluated at a range of pH 9.11, the percentage of alcohol-insoluble matter and free alkali were 24.6% and 1.6% respectively, which were within the accepted range. The antimicrobial activity of herbal soap was determined using the agar well diffusion method and exhibited antimicrobial activity against tested organisms with the highest activity against *Staphylococcus aureus*.⁸⁹

Rakesh K. Sindhu et al., (2019) conducted a study on “Formulation Development and Antimicrobial Evaluation of Polyherbal Soap.” According to this study, they formulated a polyherbal soap using Neem oil, Coconut oil, Mentha oil and Castor oil extraction. The following evaluation parameters such as color determination, pH, TFM, ethanol soluble content and Saponification value were carried out which possess a potent antimicrobial activity against various micro-

organisms such as *Escherichia coli* and *Staphylococcus aureus*. Thus, it concludes that a stable antimicrobial polyherbal soap was found to be used for daily use and did not cause any skin irritation.⁹⁰

LITERATURE REVIEW ON COMPARISON OF HERBAL SOAP WITH MARKETED SOAP

Neshtak A. L et al., (2023) conducted a study on “Development of Herbal Antibacterial Liquid Soap against bacterial skin diseases.” This study compares the formulation of antibacterial soap against Trichosan, a synthetic antibacterial soap product. For this study, Kaffir Lime Oil (KLO), King Orange Oil (KOO), Kaffir Lime Juice (KLJ), Lemon Grass Oil (LGO), *Rinacanthus nasutus* extract (RNE) and *Mangosteen* extract (MTE) was added in liquid soap and tested for the antibacterial effect of the product. Then they are tested against the following microorganisms like *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The commercial products showed a higher inhibitory effect than herbal liquid soaps, but the combination of Kaffir lime oil and king orange oil in chemical liquid soap formula shows the good synergistic effect in antibacterial properties and is better than both commercial products.⁹¹

Kandasamy Ruckman et al., (2014) conducted a study on “Formulation of Herbal Bath Soap from *Vitex negundo* Leaf Extract.” This study explains the formulation of an herbal bath soap using *Vitex negundo* Linn. (*Verbenaceae*) leaf extract and explores its antimicrobial properties. Further they are subjected to chemical characterizations such as estimation of saponification value, total fatty matter, moisture content and pH with the resulting values as 395.52 mg/mL, 70%, 6.23 % and 9.67 respectively. The antibacterial and antifungal activities of the formulated soap were performed against microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida sp.* which was found to be comparatively higher than that of the commercial antibacterial and antifungal soaps respectively as controls.⁹²

LITERATURE REVIEW ON FORMULATION OF HERBAL SHAMPOO

Rashmi C. et al., (2023) conducted a study on “Formulation and Evaluation of Herbal Anti-Dandruff Shampoo.” This study explains the preparation of the shampoo using the extracts of orange peel powder (*Citrus Aurantium Dulcis*), Curry Leaves (*Murraya Koenigii*), Aloe Vera (*Aloe Barbadensis Miller*), Reetha (*Sapindus Mukorossi*) in different proportions. The evaluation parameters of the different formulated shampoo resulted as the pH range was found to be 6.3 to 6.6; percent of solid 0.86g, 2.34g, 1.70g and 0.59g; the surface tension range were found to be 37.26 dynes/cm, 39.23 dynes/cm, 47.36 dynes/cm, 49.13 dynes/cm; wetting time were found to be 6, 10, 13 and 14 secs; the viscosity were found to be 2 min 02 secs, 8 min 28 secs, 4 min 14 secs and 5 min 34 secs and the foaming ability was found to be in the range of 15- 16ml. Thus, it clears dirt, dandruff, promotes hair growth, lustre, strengthens and darkens the hair.⁹³

Abhishek Singh et al., (2018) conducted a study on “Formulation and Evaluation of Herbal Anti-Dandruff Shampoo from Bhringraj Leaves.” In this study, the herbal anti-dandruff shampoo has been formulated using herbal ingredients such as Bhringraj Leaves extract, Lemon Grass Oil, Neem oil, Henna, Aloe Vera gel and other ingredients for base shampoo preparations. Then they were subjected to evaluation parameters such as visual inspection, pH, viscosity, solid content percentage, dirt dispersion, surface tension, foaming capacity and consistency of the foam, *Pityrosporum Ovale* strain anti-fungal activity test. The total 5 Formulations had good characteristics concerning foaming, lower pH value between the range of 5.5 to 5.9 to minimize the hair damage, percent of solids contents was found between 22-29% and a reduction in surface tension of water from 72.8 dynes/cm to 35.37 dynes/cm by the herbal shampoos is an indication of their good detergent action. Among the 5 formulations, the formulation (F4) showed strong antifungal activity (*i.e.*, maximum inhibitory region).⁹⁴

LITERATURE REVIEW ON COMPARISON OF HERBAL SHAMPOO WITH MARKETED SHAMPOO

Revansiddappa M et al., (2018) conducted a study on “Formulation and evaluation of herbal Antidandruff shampoo.” According to this study, the formulated herbal shampoo has been compared with marketed Dove shampoo. It was prepared with Ritha fruits, Liquorice stolons, Bengal gram seeds, Brahmi leaves, Greengram seeds, Banana roots, Pomegranate seeds, Hibiscus leaves, Marigold flowers and Lemon fruit extract. Different formulations are prepared which results in the Formulated shampoo (F4) is more stable than the F1, F2 and F3 formulations and is similar to marketed shampoo (Dove). The cleansing action was tested on wool yarn in grease which shows a percentage value of 33.22% obtained from herbal shampoo. The percentage cleaning action obtained for the marketed sample was 32.17%, which was marginally less than the herbal formulation. The antifungal activity of developed formulations showed positive results against the tested fungal pathogen *Candida albicans* with a maximum inhibition of 20 mm but the marketed formulation possesses lower antifungal activity with an inhibition of 19 mm as compared. Thus, it concludes that the formulated antidandruff herbal shampoo contains all the good characteristics of an ideal shampoo and it was found to be harmless, more effective and economical compared to synthetic Dove anti-dandruff shampoo.⁹⁵

Dr. Kalpana Patidar (2018) conducted a study on “Development of Polyherbal Shampoo for Antifungal Activity and its Comparison with Commercially Available Shampoo: A Research Article.” According to this study, the herbal shampoo was prepared by adding the extracts of different herbs such as pomegranate, neem, hibiscus, shikakai, reetha, curry leaves, licorice and orange peel in various proportions. All six formulations show that the shampoo was acid balanced (5.2-5.6) among which one formulation was near to skin pH. The viscosity and percent of the solid's contents were found between 21.02% and 29.01% which was easy to wash out. Among the 6 formulations, the formulation F6 showed a maximum zone of inhibition for the antifungal activity. Hence, the formulation F6 was the best formulation for treating dandruff which was compared with the marketed product, synthetic and herbal anti-dandruff shampoo containing ketoconazole.

(Nuforce shampoo) as an active ingredient and Kesh kanti (Patanjali) used as an herbal anti-dandruff shampoo. Therefore, this study concludes that the formulated product shows better antifungal activity control over dandruff than the marketed product.⁹⁶

LITERATURE REVIEW ON FORMULATION OF HERBAL HANDWASH

Zainab Irfan et al., (2023) conducted a study on “Development and Detection of Antimicrobial Properties of Polyherbal Handwash.” According to this study, the methanolic extracts of dried leaves of *Azadirachta indica*, *Ocimum gratissimum* and *Coriandrum sativum* with other ingredients like lemon juice, aloe vera, lavender oil, Hydroxypropylmethylcellulose (HPMC), triethanolamine, sodium laurylsulphate (SLS), glycerine, and methylparaben developed four batches of hand wash. Each batch was tested for stability, appearance, color, grittiness, pH, viscosity, foam height, and other physical characteristics. Then by using the agar well diffusion method, prepared polyherbal hand wash was tested against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans*. All formulations were found to be greenish in colour, mild lavender fragrant, sleek, pellucid, with no grittiness, and maintain homogeneous form respectively with a pH range of 6 to 7. They also demonstrate that all formulations show no irritation or redness and have no chance for skin sensitivity. Among the total 4 formulations, the F3 batch shows good foam height, good retention time with a stable for 6 min and has shown good antimicrobial activity against five different microorganisms.⁹⁷

Salgaonkar Snehal et al., (2015) conducted a study on “Development of anti-fungal herbal Hand wash gel.” This study aims to evaluate the anti-fungal efficacy of the formulated herbal hand wash liquid containing Lemongrass and Bermuda grass extracts. The acetone extract of *Cymbopogon citrates* (Lemongrass) and *Cynodon dactylon* (Bermuda grass) are used for the formulation of handwash. It was then undergoing for the antifungal activity by agar-cup method technique against *Aspergillus niger* with Mueller-Hinton agar as a culture medium. The results prove that the extract of lemon grass and Bermuda grass is active against *Aspergillus niger*. Hence it was encouraging to be used in the preparation

of herbal hand wash gel with little or no side effects by providing safe and healthier living through germ-free hands. Although the removal is not 100% but a major number can be reduced.⁹⁸

LITERATURE REVIEW ON COMPARISON OF HERBAL HAND WASH WITH MARKETED HAND WASH

Souvik Giri et al., (2020) conducted a study on "Comparison and Evaluation of Antimicrobial Activity of *Mimosa pudica*, *Azadirachta Indica* and Citrus Limon in Aqueous Based Hand Wash with Marketed Herbal Products." This study explains the formulation of herbal handwash using ethanolic extracts of *Mimosa pudica* (Touch me not), *Azadirachta indica* (Neem), and lemon extract which has been compared with the marketed herbal product. Two formulations of herbal handwashes were prepared and evaluated. Both the formulation appears greenish brown and greenish yellow color with no grittiness and smooth on application; the pH was found to be 6.3 and 6.2; the viscosity was found to be 64 and 72 CPS. Then the antimicrobial activity was tested against the microorganisms which resulted in S-1 showing significant antimicrobial activity and the S-2 showed a little higher activity than the S-1. After that the S-2 was compared with the standard marketed product (Patanjali Anti-bacterial herbal hand wash/Sri Tattva Klean up hand wash). Hence this study concluded that herbal hand wash provides a superior zone of inhibition to protect against skin pathogens with an effective and safe alternative to existing marketed synthetic hand wash.⁹⁹

CHAPTER-3
AIMANDOBJECTIVESOFWORK

CHAPTER–3

AIM AND OBJECTIVES OF WORK

AIM OF THE STUDY:

This study aims to find the Formulation and Evaluation of various antimicrobial cosmetic products using extracts of *Sphagneticola trilobata*.

OBJECTIVES OF THE WORK:

PRIMARY OBJECTIVE:

To identify the phytochemical screening and antimicrobial activity of *Sphagneticola trilobata* using its extracts

SECONDARY OBJECTIVE:

To formulate & evaluate the extracts of *Sphagneticola trilobata* into herbal cosmetic products, [soap, shampoo, handwash].

CHAPTER – 4
EXPERIMENTALWORK

CHAPTER – 4

EXPERIMENTALWORK

MATERIALSANDMETHODS:

Sphagneticolatrilobata:

MACROSCOPICALCHARACTERS:

Leavescolour: Darkgreenaboutandpaler below.

Flowercolour: Yellow.

Odour: Characteristicandadmissibleodour¹⁰⁰

MORPHOLOGICALFEATURES:

Partsused: Wholepartsoftheplant.

Leaves: The leaves are simple, opposite, sessile or borne on very short petioles, slightlyfleshy, mostlyhairless, glossy, 4-9cmlongand2-5cmwide; laminaisusually3-lobedandwithirregularlytoothedmargins, Colourisdark green above, paler below. ¹⁰⁰

Stem: The stems are rounded, solid, green or reddish, somewhat hairy to almosthairless. Theygrowupto4mlongandregularlydevelopadventitious roots at their joints. Short, semi-upright flowering branches are produced off these creeping stems¹⁰⁰

Root: The root system is shallow and fibrous, allowing the plant to anchor itself and absorb water and nutrients from the soil. These roots spread horizontallynear the surface of the soil, enabling the plant to propagate and establish itself effectively. However, they may not penetrate deeply into the soil¹⁰⁰

Flower: Theinflorescencesarebrightyellowtoorange-yellowdaisy-like flowerheadsbronesinglyonerectstalks¹⁰⁰

COLLECTION OF SAMPLES:

Sphagneticola trilobata was collected from in and around area of Navalur-603103 and from Sathyabama Institute of Science and Technology (SIST) Chennai - 603119 and authenticated from Siddhars research institute, Arumbakkam, Chennai - 6001 1006. (Form no: PCOG002- ACF) [Annexure-1]

PREPARATION OF EXTRACTION:

The whole part of plant *Sphagneticola trilobata* was collected as shown in Figure 4.1. The leaf, flower, stem, root, parts are segregated separately as shown in Figure 4.2, 4.3, 4.4. They are washed twice with hot water for 10 mins and shade dried for 7 days as shown in Figure 4.5. The dried plant parts were stored in a zip lock cover as shown in figure 4.6. The stored shade dried plant parts are powdered and were passed through sieve #40 mesh size and stored as shown in figure 4.7.

The sieved powder is further involved for the extraction process. The extraction was done with three different solvents, they are Distilled water, Ethanol, and Ethyl acetate by maceration technique and they are filtered after 15 days. The filtered extract was tested against Gram positive bacteria (*Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli*) and against fungi (*Candida albicans*) by agar well diffusion method (25µl, 50µl, 75µl, 100µl). The well was filled with the extract in the petri dish plate over the Mueller Hinton Agar base. After 24 hours the zone of inhibition is observed for anti bacterial and after 48 hours the zone of inhibition is observed for antifungal activity respectively.



Figure 4.1 Whole plant of *Sphagneticola trilobata*



Figure4.2 Leaf are segregatedseperately



Figure4.5 Thecollectedpart
of*Sphagneticolatrilobata*
shadedry



Figure4.3Flower aresegregatedseperately.



Figure4.6Thecollectedpart
of*Sphagneticolatrilobata*
stored.



Figure 4.4 Stem and Root are segregated
Seperately.



Figure 4.7 The collected part
of*Sphagneticolatrilobata's*
powder.

DISTILLED WATER EXTRACT OF *S. trilobata*:

The crude powdered drug 100g was added with 500ml of Distilled water and they are macerated for 15 days by covering it with aluminium foil in the beaker and they are labelled. After 15 days they are filtered with the help of filter paper. The filtered extract was further taken for the evaluation of antimicrobial activity as shown in figure 4.8 Formulation and evaluation was performed further.

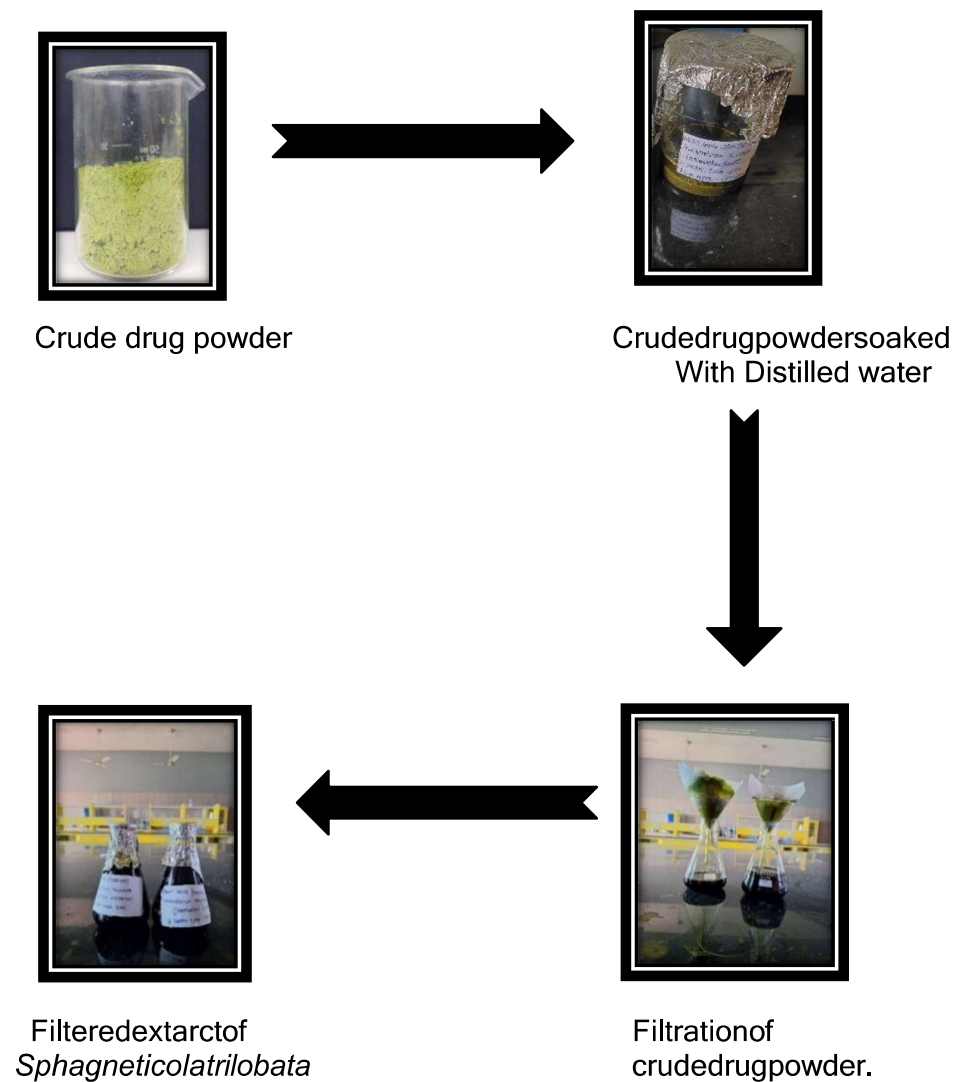


Figure 4.8 The process of obtaining distilled water extract from *S. trilobata*.

ETHANOLEXTRACT OF *S.trilobata*:

The crude powdered drug 100g was added with 500ml of Ethanol and they are macerated for 15 days by covering it with aluminium foil in the beaker and they are labelled. After 15 days they are filtered with the help of filter paper. The filtered extract was further taken for the evaluation of antimicrobial activity as shown in figure 4.9. Formulation and evaluation was performed further.

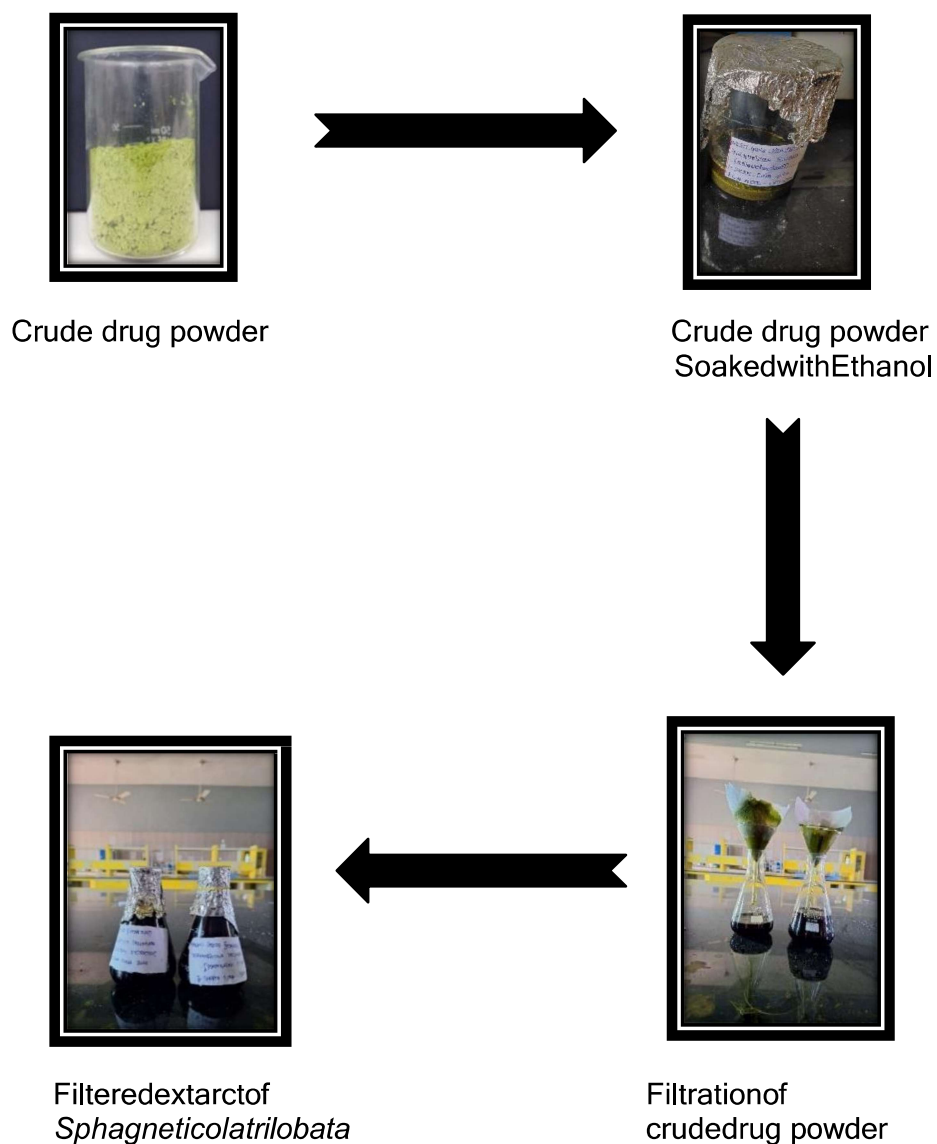


Figure 4.9 The process of obtaining ethanol extract from *S. trilobata*

ETHYLACETATEEXTRACTOFS.*trilobata*:

The crude powdered drug 100g was added with 500ml of Ethyl Acetate and they are macerated for 15days by covering it with aluminium foil in the beaker and they arelabelled. After 15 days they are filtered with the help of filter paper The filteredextractwasfurthertakenfortheevaluationofantimicrobialactivityasshown in figure 4.10 Formulation and evalutaion was performed further

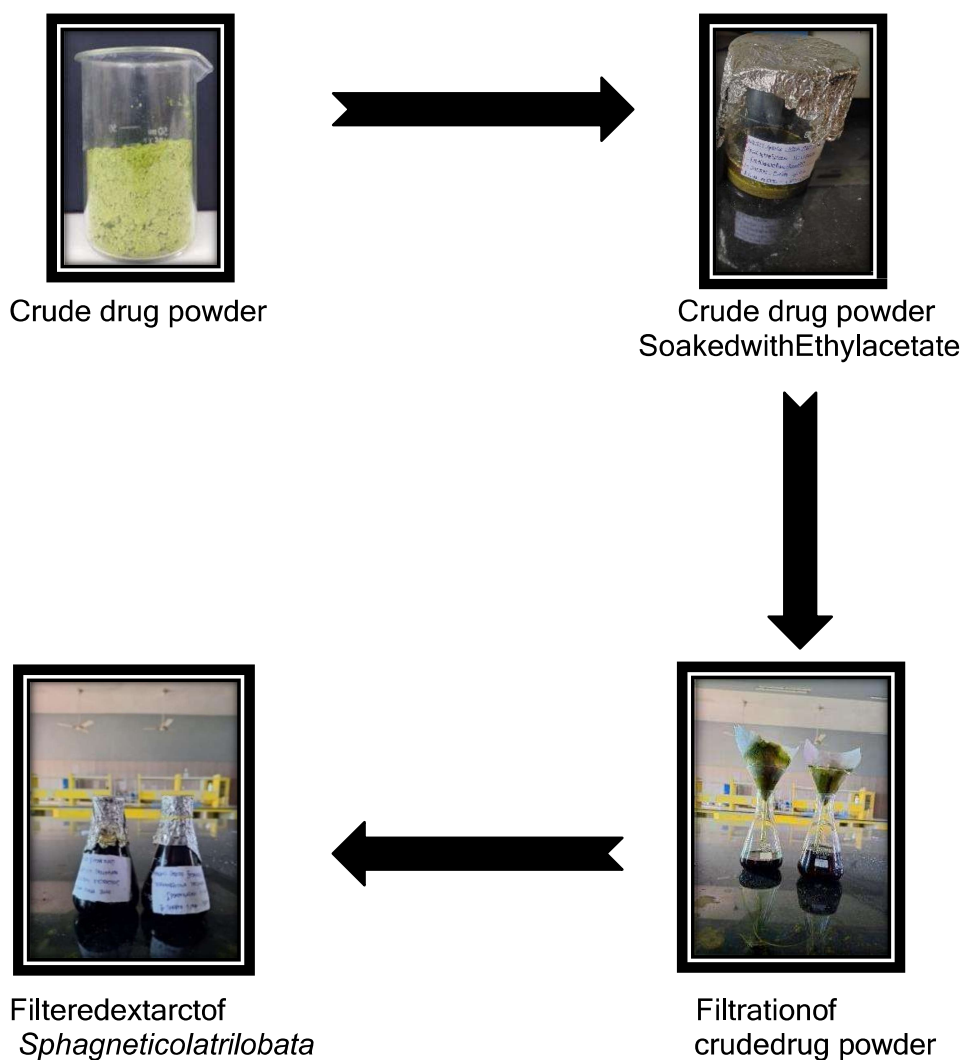


Figure4.10The processof obtaining ethylacetateextract from *S.trilobata*

4.8 COMPARISON OF THREE SOLVENT EXTRACTS OF *S. trilobata*:

Among the three solvents, ethyl acetate extract had the potent anti- microbial activity. Thus, they are further considered for the formulation and evaluation of herbal cosmetic preparations.

AGAR WELL DIFFUSION:

The distilled water, ethanol, ethyl acetate filtered extracts of *s. trilobata* are given for the evaluation of antimicrobial activity by agar well diffusion method.

Principle:

The agar diffusion assay is a widely used method for determining the antimicrobial activity of plants or microbial extracts (Chelliah, R et al 2022).¹⁰¹ The agar diffusion technique is commonly used for determination of MIC in solid media, and it involves the application of extracts solutions of different concentrations to wells placed on the surface of or punched into agar plates seeded with the test bacterial strain (Boyan Bonev et al 2008)¹⁰²

Procedure:

1. Prepare Mueller Hinton agar plates (pH 7.2- & 4-mm depth).
2. Inoculate the test organisms (*Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*) prepared in a sterile tube by streaking the loop in a back-and-forth motion over the entire agar surface, or by using a sterile cotton swab.
3. Punch a hole with a diameter of 6 to 8 mm aseptically with a sterile cork borer or a well puncher.
4. Fill the hole with a volume (20-100 μ L) of the test extract solution at the desired concentration.
5. Incubate the agar plates under suitable conditions depending upon the test microorganism. In case of Pathogenic species, the plates should be incubated for 24 hours for antibacterial activity and 48 hours for antifungal activity.
6. After the incubation period, measure the zone of inhibition, which includes the wells' diameter, to identify the presence or absence of antimicrobial activity.

Sample preparation:

The plant extract was dissolved in DMSO and mixed properly to get a uniform suspension, the final concentration of the plant extract was as 65 µg/ml.

Organism:

Organism used in anti-microbial activity *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*.

Materials required:

- Sterile micropipette
- Sterile filtered micropipette tips
- Sterile cork borer/well puncher
- Sterile cotton swab
- Disposable Petri plates
- Sterile media
- Sterile distilled water
- Standard drug-drug control

PHYTOCHEMICAL SCREENING OF PLANT EXTRACTION:

Phytochemicals are regarded as secondary metabolites. The medicinal value of plants depends on numerous active compounds present in the plant which are helpful in improving the therapeutic activity of plant. Qualitative phytochemical screening of *Sphagneticol trilobata* (L.) Pruski., studied with plant extracts obtained from distilled water, ethanol, and ethyl acetate. The phytochemical screening was performed for all 3 solvent extracts and it is observed for presence and absence of secondary metabolites.

Test for Alkaloids:

The powder plant material was boiled with 1ml of dilute sulphuric acid and filtered. The filtrate was used for the following test.¹⁰³

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.¹⁰³

b) Wager's Test

To the filtrate, few drops of Wager's reagent was added and shaken well. Formation of reddish-brown precipitate was formed showed the presence of alkaloids.¹⁰³

Test for Flavonoids:

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 Colour was formed the presence of flavonoids¹⁰³

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 flavonoids.¹⁰³

Test for Tannins:

1 ml of crude extract was mixed with 2 ml of 2% FeCl₃ solution. A blue green or black coloration indicates the presence of phenols and tannins.¹⁰³

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 saponins¹⁰³

Test for Steroids:

Liebermann Burchard Test

Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the slides of the test tube and observed for the formation of a brown ring at the junction of two layers. Formation of green coloration of the upper layer indicates the presence of steroids¹⁰³

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 layer. A reddish –brown Coloration of the interface was formed to show positive results for the Presence of terpenoids.¹⁰³

Test for Glycosides:**a) Keller Kelliani Test**

5ml of extract was treated with 2ml of glacial acetic acid containing one Drop of ferric chloride solution. This was underplayed with concentrated H_2SO_4 . A greenish ring may form just gradually throughout thin layer.¹⁰³

Test for Quinones:**a) Quinones Test**

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow Precipitate.¹⁰³

Test for Proteins:**a) Biuret Test**

Test solution was treated with 10% sodium hydroxide solution and two drops of 1% copper sulphate solution and observed for the formation of violet/ pink colour¹⁰³

Test for Free amino acids:**a) Ninhydrin Test**

Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acid.¹⁰³

Test for Carbohydrate:**a) Benedict's test**

Test solution was mixed with few drops of Benedict's reagent and boiled in

waterbath,observedfortheformationofreddishbrownprecipitatetoshowa positive result for the presence carbohydrate.¹⁰³

FORMULATIONOFANTIFUNGALHERBALCOSMETICS:

FORMULATIONOFANTIFUNGALHERBALSOAP:

TheAntifungalherbalsoapwaspreparedbymeltandpourmethod.Theglycerine soap base was melted by double heat method. To this melted base the ethyl acetate extract of *Sphagneticola trilobata* was added and mixed. The rose essential oil was added for enhancement of odour and they are poured to the base after brushing the soapmouldwiththeglycerinebaseandtheytheirallowedtosettleaftertappingtoavoid airbubbles.After15minutesthesoapisremovedfromthemouldasshowninfigure 4.11furthertheyarepacked&labelled.Furtherevaluation parameterisperformed.

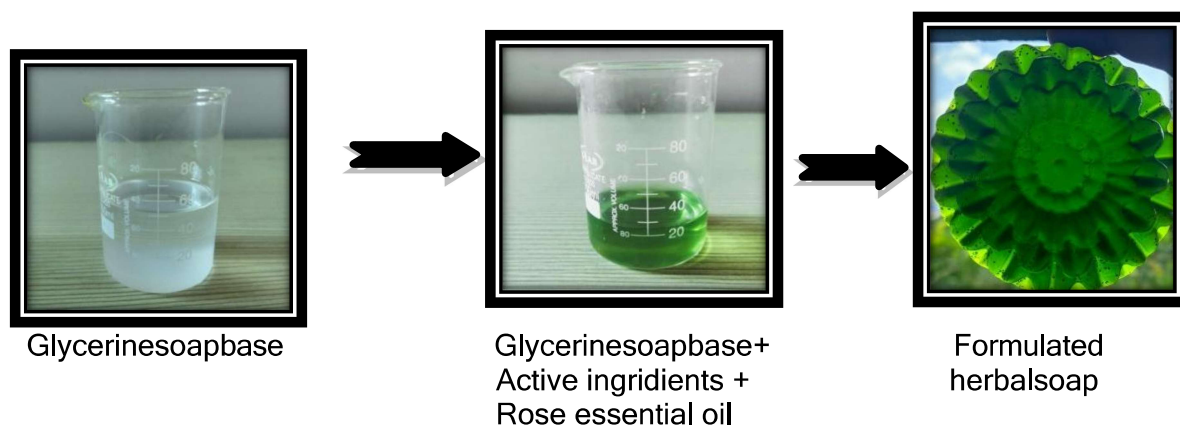


Figure4.11FormulationofAntifungalHerbalsoup

Table 4.1 Formulation of Herbal Soap

INGREDIENTS		QUANTITY			
		F1	F2	F3	F4
Glycerine soap base	Humectant	50g	50g	50g	50g
<i>S. trilobata</i> ethyl acetate extract	Antifungal	5g	10g	15g	20g
Rose essential oil	Perfume	5 drops	5 Drops	5 drops	5 drops

Evaluation Parameters for Herbal Soap:**Organoleptic Evaluation:**

Using our eyes and nose, we evaluate the soaps colour, clarity, and odour against a white background.¹⁰⁴

Measurement of pH:

1 gm of soap sample was taken & was dissolved in 10 mL of water. Using a digital pH meter, the pH of each of the generated formulations was assessed.¹⁰⁴

Foam Retention:

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.¹⁰⁴

Foam Height:

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.¹⁰⁴

Determination of Percentage of Free Alkali:

Measure 10 g 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.¹⁰⁴

Alcohol Insoluble Matter:

A 5 g sample was placed in a conical flask. 50 mL of warm methanol 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 measured¹⁰⁴

Total Fatty Matter:

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 formula¹⁰⁴

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

Moisture Content:

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%.¹⁰⁴

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

Saponification:

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.¹⁰⁴

Saponification value

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

Foam Forming Ability:

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.¹⁰⁴

Skin Irritation Test:

Wash your skin thoroughly, then apply the soap sample to your skin and look for any irritation, burning, itching, or any other symptoms.¹⁰⁴

Antifungal Activity

The Antifungal Activity was evaluated by the agar well diffusion technique.

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 in different concentration of 25ml, 50ml, 75ml, 100ml
- 100 µL of sample were pipetted out in respective wells.
- Control wells were maintained: positive control
Negative control distilled water
- Incubate the plates at 30°C for 48 hrs.
- After incubation, zone of inhibition (ZOI) around the wells were measured in diameter (mm).

FORMULATION OF ANTIFUNGAL HERBAL SHAMPOO:

The herbal shampoo was prepared by using primary emulsion method.

Beaker 1: The ethyl acetate plant extract and castor oil were mixed together as oil part.

Beaker 2: The polyethyleneglycol (PEG), glycerine and methylparaben were mixed together as water part.

Now pour the oil phase mixture (beaker 1) to water phase mixture (beaker 2) along with a continuous stirring. Acacia and tragacanth were used as gum which help to form soap in water (o/w). To this solution add the extract of Shikakae (Acacia concinna) and Soap Nut (Sapindus mukorossi) and mix well together. Finally, the pH of the solution was adjusted by adding sufficient quantity of 1% citric acid solution. Few drops of essential oil were also added to impart a aroma to the prepared shampoo as shown in

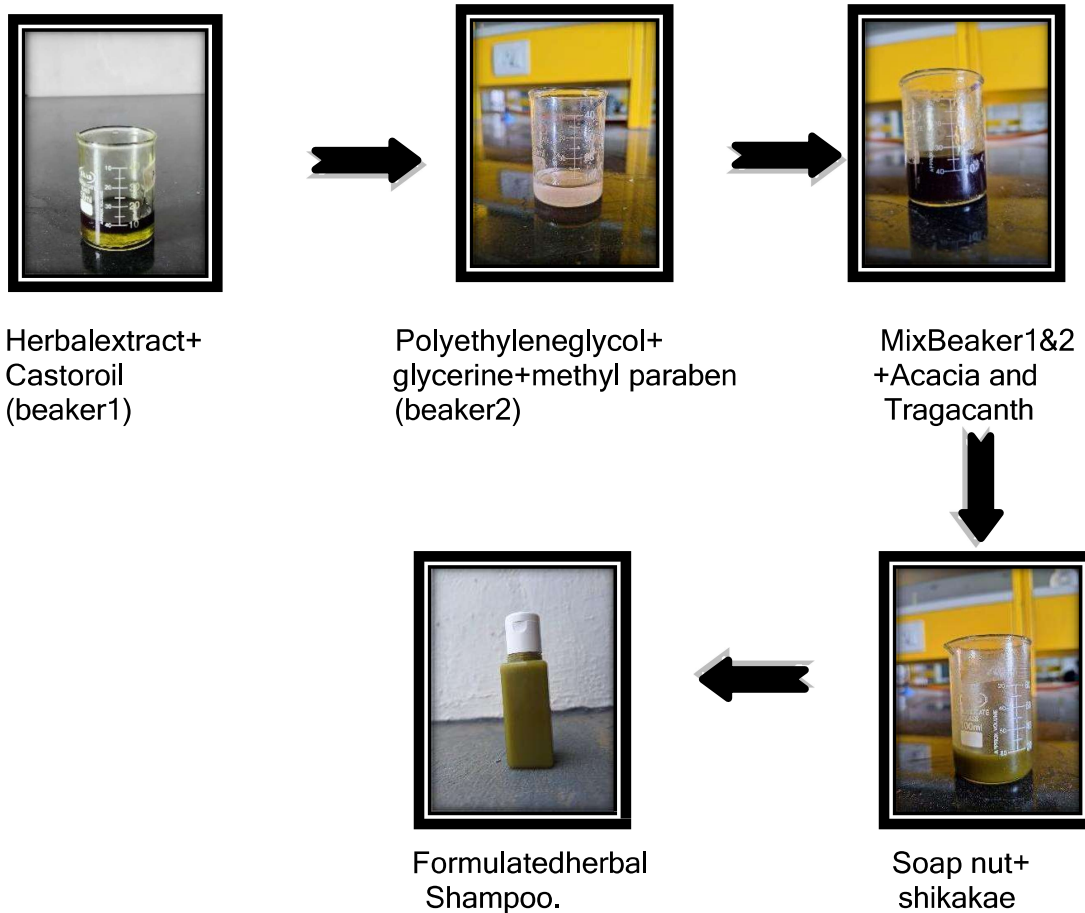


Figure 4.12 Formulation of Antifungal Herbal Shampoo

Table 4.2 Formulation of Antifungal Herbal Shampoo

INGREDIENTS	QUANTITY			
	F1	F2	F3	F4
DRUG	2ml	4ml	6ml	8ml
CASTOR OIL	12.75ml	12.75ml	12.75ml	12.75ml
POLYETHYLENE GLYCOL	6ml	6ml	6ml	6ml
GLYCERINE	1.5ml	1.5ml	1.5ml	1.5ml
METHYL PARABEN	0.75ml	0.75ml	0.75ml	0.75ml
ACACIA	2.25ml	2.25ml	2.25ml	2.25ml
TRAGACANTH	1.5ml	1.5ml	1.5ml	1.5ml
ESSENTIAL OIL	q.s	q.s	q.s	q.s
CITRIC ACID	1.25ml	1.25ml	1.25ml	1.25ml
SHIKAKAI	2.55g	2.55g	2.55g	2.55g
SOAPNUT	1.25g	1.25g	1.25g	1.25g
DISTILLED WATER	q.s	q.s	q.s	q.s

Evaluation Parameters for Herbal Shampoo:

To evaluate the prepared formulation, quality control tests including visual assessment and physicochemical controls such as pH, density and viscosity were performed. Also, to assure the quality of products, specific tests for shampoo formulations including the determination of dry residue and moisture content, total surfactant activity, salt content, surface tension, thermal and mechanical stability

and detergency tests were carried out. The results were compared with marketed formulation.¹⁰⁵

Physical appearance/visual inspection:

The formulations prepared were evaluated in terms of their clarity, foam producing ability and fluidity.¹⁰⁵

Determination of pH:

The pH of 10% shampoo solution in distilled water was determined at room temperature 25°C.¹⁰⁵

Determine percent of solids contents:

A clean dry evaporating dish was weighed and added 4 grams of shampoo to the evaporating dish. The dish and shampoo were weighed. The exact weight of the shampoo was calculated only and put the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated.¹⁰⁵

Rheological evaluations:

The viscosity of the shampoo was determined by using Brookfield Viscometer (Model DV-I Plus, LV, USA) set at different spindle speeds from 0.3 to 10 rpm. The viscosity of the shampoos was measured by using spindle T95. The temperature and sample container's size were kept constants during the study.

¹⁰⁵**Cleaning action:**

5 grams of wool yarn were placed in grease, after that it was placed in 200 ml. of water containing 1 gram of shampoo in a flask. Temperature of water was maintained at 35°C. The flask was shaken for 4 minutes at the rate of 50 times a minute. The solution was removed and sample was taken out, dried and weighed. The amount of grease removed was calculated by using the following equation:

$$DP = 100(1 - T/C)$$

In which, DP is the percentage of detergency power, C is the weight of sebum in the control sample and T is the weight of sebum in the test sample¹⁰⁵

Foaming ability and foam stability:

Cylinder shake method was used for determining foaming ability. 50 ml of the 1% shampoo solution was put into a 250 ml graduated cylinder and covered the cylinder with hand and shaken for 10 times. The total volume of the foam contents after 1 minute shaking were recorded. The foam volume was calculated only. Immediately after shaking the volume of foam at 1 minute intervals for 4 minutes

were recorded.¹⁰⁵

FORMULATION OF ANTIFUNGAL HERBAL HANDWASH:

Hand wash was prepared by adding the filtrate of ethyl acetate plant extract in a beaker. To this filtrate sodium lauryl sulphate, acacia, glycerin, methyl paraben, rose oil were added and mixed well together. Now make up to the desired volume with the help of distilled water as shown in figure 4.14

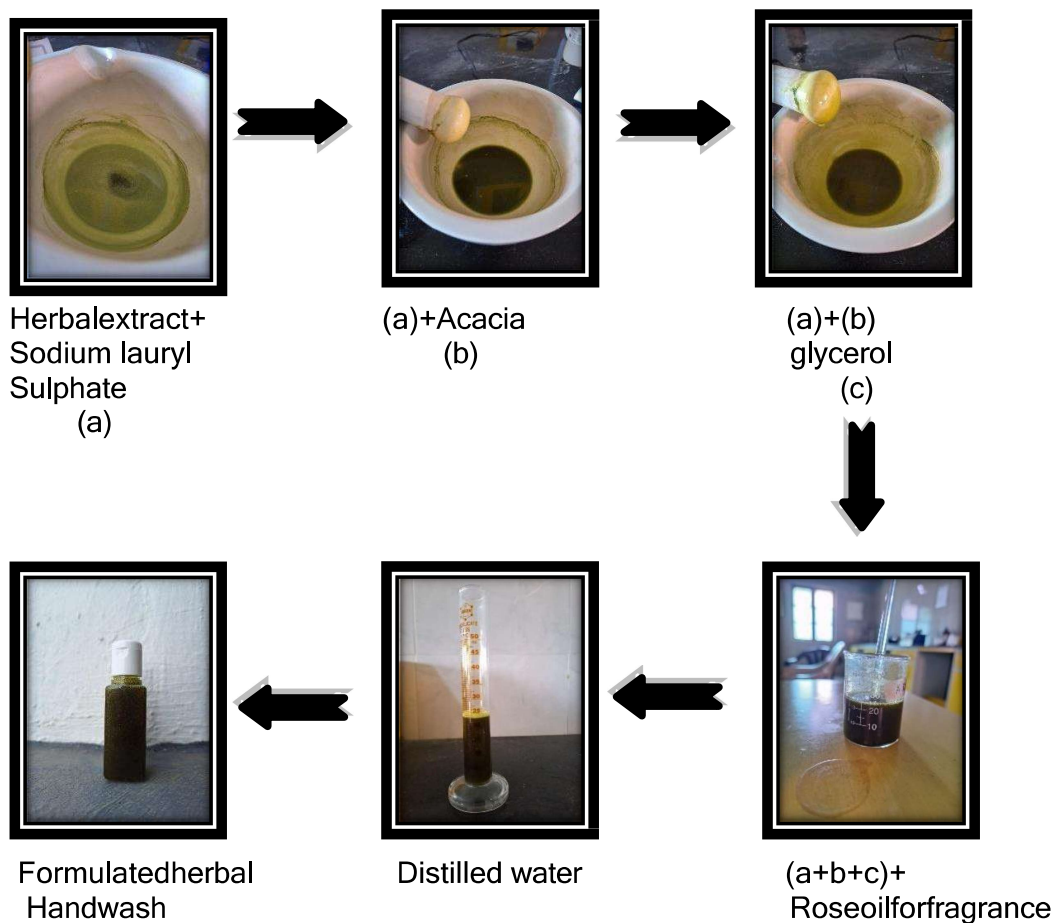


Figure 4.14 Formulation of Antifungal Herbal Handwash

Table 4.3 Formulation of Antifungal Herbal Handwash

INGREDIENTS	QUANTITY			
	F1	F2	F3	F4
Drug	2ml	4ml	6ml	8ml
Glycerol	7.5ml	7.5ml	7.5ml	7.5ml
Acacia	1.25g	1.25g	1.25g	1.25g
SLS	1.5g	1.5g	1.5g	1.5g
Methylparaben	0.07g	0.07g	0.07g	0.07g
Rose oil	q.s	q.s	q.s	q.s
Distilled water	q.s	q.s	q.s	q.s

Evaluation Parameters for Herbal Hand Wash**pH:**

Before every single pH measurement or before any series of uses pH meter must be calibrated. After calibration rinse electrode and submerge it in the tested solution.¹⁰⁶

Viscosity:

The viscosity of hand wash was determined by using Brookfield viscometer. Measured quantity of herbal hand wash was taken into a beaker and the tip of viscometer was immersed into the hand wash gel and the viscosity was measured in triplicate¹⁰⁶

Odour:

The odour was perceived by sensing the formulation.¹⁰⁶

Foam Height:

1gm of sample of Poly Herbal Gel Based hand wash was taken and dispersed in 50 ml distilled water. Then, transferred it into 500 ml stoppered measuring cylinder; volume was made up to 100 ml with water. 25 strokes were given & stand till aqueous volume measured upto 100 ml & measured the foam height; above the aqueous volume.¹⁰⁶

Foam Retention:

50 ml of the Poly Herbal Gel Based hand wash was taken into a 250ml graduated cylinder and shaken 10 times. The volume of foam at 1-minute intervals for 4 minutes was recorded. Foam retention should remain stable for at least 5 minutes.¹⁰⁶

Spreading Coefficient:

Two glass slides were taken of known dimensions and an excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between. This slide and another glass slide having the dimension of fixed ground slide. A 500g weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. A shorter interval indicates better Spreadability was calculated using the following formula:

$$S = ML/T$$

Where, S = Spreadability, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide T = Time (in sec.) Taken to separate the slide completely each other Skin Irritation The formulation was applied on skin and left for 30 min¹⁰⁶

CHAPTER – 5
RESULTANDDISCUSSION

CHAPTER – 5

RESULTS AND DISCUSSION

EVALUATION OF PHYTOCHEMICAL SCREENING OF *Sphagneticola*

trilobata:

- Phytochemical analysis reveals the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, phenolics, tannins, proteins, free amino acids and carbohydrates whereas glycosides, quinones, phlobatannins and oxalate are absent in whole plant of *Sphagneticola trilobata* using the solvent distilled water as shown in the figure 5.1
- Phytochemical analysis reveals the presence of alkaloids, flavonoids, saponins, steroids, phenolics, tannins, proteins, free amino acids and carbohydrates whereas terpenoids, glycosides, quinones, phlobatannins and oxalate are absent in whole plant of *Sphagneticola trilobata* using the solvent ethanol as shown in the figure 5.2
- Phytochemical analysis reveals the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, phenolics, tannins and proteins whereas quinones, phlobatannins, oxalate, free amino acids and carbohydrates are absent in whole plant of *Sphagneticola trilobata* using the solvent ethyl acetate as shown in the figure 5.3
- Then the phytochemical screening of different solvent extract (i.e. distilled water, ethanol, ethyl acetate) of whole plant of *Sphagneticola trilobata* was compared and analyzed as shown in the table 5.1
- Due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols, saponins and alkaloids present in ethyl acetate extract of *Sphagneticola trilobata* showing antimicrobial activity. The ethyl acetate extract is further considered for the formulation of herbal cosmetics.

TABLE 5.1 Qualitative Phytochemical Screening of different solvent extracts of *Sphagneticol trilobata*

PHYTOCHEMICAL TESTS	ETHANOL	DISTILLED WATER	ETHYL ACETATE
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Terpenoids	-	+	+
Steroids	+	+	+
Glycosides	-	-	+
Tannis & Phenol	+	+	+
Quinones	-	-	-
Phlobatannins	-	-	-
Oxalate	-	-	-
Proteins	+	+	+
Free amino acids	+	+	-
Carbohydrates	+	+	-



FIGURE 5.1 Phytochemical screening for the distilled water extract of *Sphagneticol trilobata*



FIGURE 5.2 Phytochemical screening for the ethanol extract of *Sphagneticol trilobata*



FIGURE 5.3 Phytochemical screening for the ethyl acetate extract of *Sphagneticol trilobata*

**ANTIMICROBIAL ZONE OF INHIBITION BY VARIOUS EXTRACTS OF
Sphagneticol trilobata:
 ANTIMICROBIAL ACTIVITY OF DISTILLED WATER EXTRACT:**

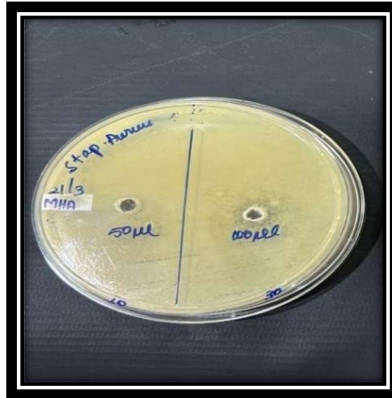


FIGURE 5.4 Zone of inhibition
Staphylococcus aureus

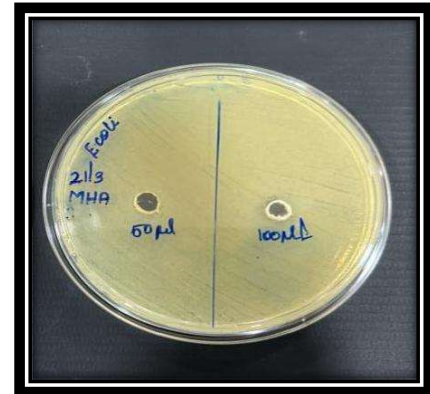


FIGURE 5.5 Zone of inhibition of
Escherichia coli

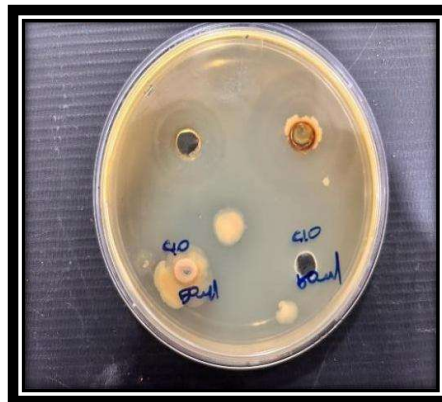


FIGURE 5.6 Zone of inhibition of *Candida albicans*

The distilled water extract of *S. trilobata* undergoes the antimicrobial activity against the tested pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) with a standard positive control (i.e. antibiotic) and with a negative control (i.e. distilled water) at a concentration of 50 µl, 100 µl respectively by agar well diffusion method.

ANTIMICROBIALACTIVITYOFETHANOLEXTRACT:

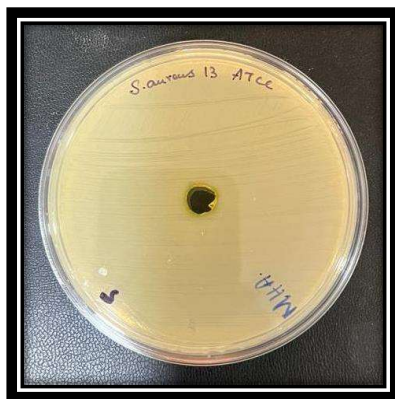


FIGURE 5.7 Zone of inhibition
Staphylococcus aureus

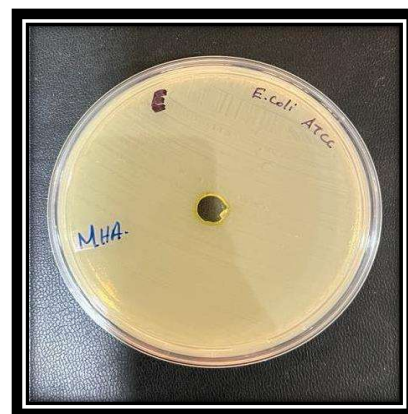


FIGURE 5.8 Zone of inhibition of
Escherichia coli



FIGURE 5.9 Zone of inhibition of *Candida albicans*

The ethanol extract of *S. trilobata* undergoes the antimicrobial activity against the tested pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) with a standard positive control (i.e. antibiotic) and with a negative control (i.e. distilled water) at a concentration of 25 μ l, 50 μ l, 75 μ l, 100 μ l respectively by agar well diffusion method.

ANTIMICROBIALACTIVITYOFETHYLACETATE EXTRACT:

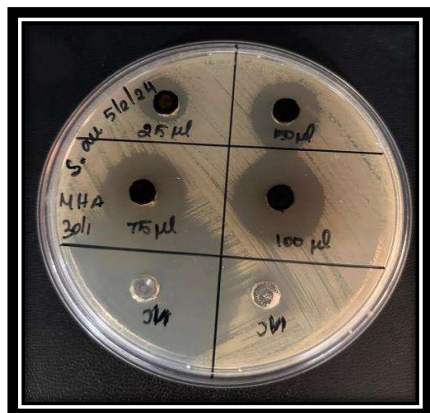


FIGURE5.10Zoneofinhibition
of *Staphylococcus aureus*

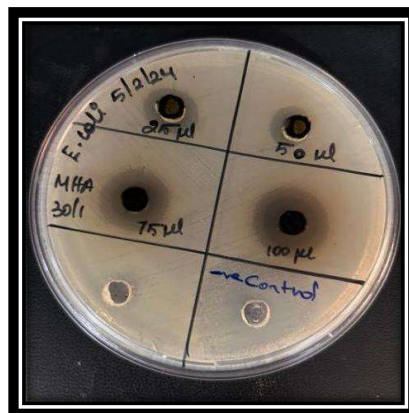


FIGURE5.8Zoneofinhibition
of *Escherichia coli*

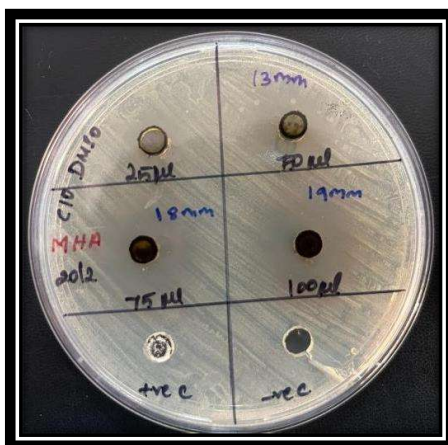


FIGURE5.12Zoneofinhibitionof *candida albicans*

The ethyl acetate extract of *S. trilobata* undergoes the antimicrobial activity against the tested pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) with a standard positive control (i.e. antibiotic) and with a negative control (i.e. distilled water) at a concentration of 25 µl, 50 µl, 75 µl, 100 µl respectively by agar well diffusion method.

COMPARATIVE ANALYSIS OF DIFFERENT SOLVENT EXTRACT:

The ethyl acetate extract showed antimicrobial activity against tested pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*). Ethyl acetate shown a maximum antibacterial activity with a zone of inhibition of 13 mm, 17 mm, 21 mm and 24 mm at a concentration of 25 µl, 50 µl, 75 µl, 100 µl respectively in *Staphylococcus aureus* (gram +ve bacteria) as shown in the figure 5.10. Secondly *Escherichia coli* (gram -ve bacteria) shows a maximum antibacterial activity with a zone of inhibition of 14 mm, 10 mm, 18 mm and 19 mm at a concentration of 25 µl, 50 µl, 75 µl, 100 µl respectively as shown in the figure 5.11. It also shows a maximum antifungal activity with a zone of inhibition of 13 mm, 18 mm and 19 mm at a concentration of 50 µl, 75 µl, 100 µl respectively whereas at 25 µl it shows nil activity in *Candida albicans* as shown in the figure 5.12. But the ethanol and distilled water extract does not show the antimicrobial activity against the all tested bacterial pathogens and fungi (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) as shown in the figure 5.4, 5.5, 5.6, 5.7, 5.8, 5.9. Thus, the comparative analysis of the antimicrobial activity of different solvents of *Sphagneticola trilobata* reveals that the ethyl acetate extract shown significant inhibitory action against all tested bacterial pathogens and fungi as shown in the figure 5.13

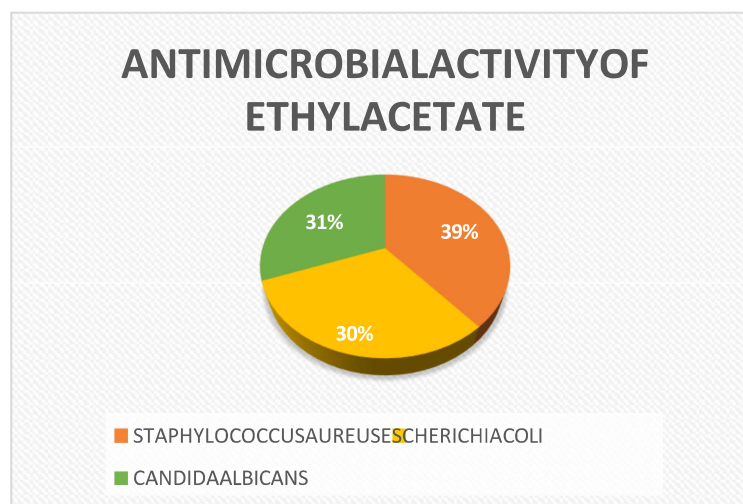


FIGURE 5.13 Pie chart on antimicrobial activity of ethyl acetate extract of *Sphagneticola trilobata*

EVALUATION OF ANTIFUNGAL HERBAL COSMETICS:

ANTIFUNGAL HERBAL SOAP:

The physical evaluations of herbal soap formulations F1, F2, F3 and F4 include color, odour, pH, foam retention, foam height, percentage free alkali, alcohol-insoluble matter, total fatty matter, moisture content, foam-forming ability and skin irritability were measured. For F1; it is transparent in appearance with a pleasant odour. The pH level was 4.3, the foam retention was 3.2 cm, the foam height was 3 cm, the percentage of free alkali was 2.3%, alcohol insoluble matter was 15.7%, the total fatty matter was 60%, the moisture content was 12.8 %, the foam forming ability was 9.1 cm, and there was no skin irritation. For F2; it is mild green in appearance with a pleasant odour. The pH level was 4.6, the foam retention was 3.2 cm, the foam height was 2.8 cm, the percentage of free alkali was 2.7%, alcohol insoluble matter was 16.5%, the total fatty matter was 62%, the moisture content was 13.2%, the foam forming ability was 7.6 cm and there was no skin irritation. For F3; it is dark green in appearance with a pleasant odour. The pH level was 5.5, the foam retention was 3.5 cm, the foam height was 2.5 cm, the percentage of free alkali was 2.3%, alcohol insoluble matter was 17.4%, the total fatty matter was 60 %, the moisture content was 13%, the foam forming ability was 8 cm and there was no skin irritation. For F4; it is dark green in appearance with a pleasant odour. The pH level was 5.1, the foam retention was 3.4 cm, the foam height was 3.3 cm, the percentage of free alkali was 2.2%, alcohol insoluble matter was 16.9%, the total fatty matter was 63.4%, the moisture content was 13.8%, the foam forming ability was 10.4 cm, and there was a mild skin irritation as shown in the table 5.2. Then the 4 formulations were further sent for antifungal activity in Col. Dr. Jeppiaar Research Park in Sathyabama Institute of Science & Technology. Among them the F3 shows the best physicochemical properties and maximum antifungal activity on *Candida albicans* which showed around 13 mm zone of inhibition in agar well of 75 µl and 100 µl concentration and shows nil activity at a concentration of 25 µl and 50 µl respectively as shown in the figure 5.14.



FIGURE 5.14 Zone of inhibition of *Candida albicans* in herbal soap.
TABLE 5.2 Evaluation parameters of formulated antifungal herbal soap

EVALUATION PARAMETERS	F1	F2	F3	F4
Colour	Transparent	Mild green	Dark green	Dark green
Odour	Pleasant	Pleasant	Pleasant	Pleasant
pH	4.3	4.6	5.5	5.1
Foam Retention (min)	3.2	3.2	3.5	3.4
Foam height (cm)	3	2.8	2.5	3.3
Percentage free alkali (%)	2.3	2.7	2.3	2.2
Alcohol insoluble Matter (%)	15.7	16.5	17.4	16.9
Total fatty matter (%)	60	62	60	63.4
Moisture content (%)	12.8	13.2	13	13.8
Foam forming ability (%)	9.1	7.6	8	10.4
Skin irritation	No	No	No	Very mild
Zone of inhibition (mm) at 75 µl concentration	12	12.5	13	12.8

ANTIFUNGAL HERBAL SHAMPOO:

The physical evaluations of herbal shampoo formulations F1, F2, F3 and F4 include color, odour, viscosity, pH, stability, foam height, foam retention and skin irritability were measured. For F1; it is green in appearance with a pleasant odour. The viscosity was found to be 48 cps, pH level was 4.64, percentage of solid contents was 22.11%, the foam height was 8cm, the foam retention was 2.3 mins, cleaning action was 19.81%, foam ability and foam stability was 22 ml and there was no skin irritation. For F2; it is mild green in appearance with a pleasant odour. The viscosity was found to be 50 cps, pH level was 5.18, percentage of solid contents was 20.32%, the foam height was 7.5cm, the foam retention was 2.8mins, cleaning action was 24.21%, foam ability and foam stability was 19.8 ml and there was no skin irritation. For F3; it is green in appearance with a pleasant odour. The viscosity was found to be 57 cps, pH level was 5.59, percentage of solid contents was 28.26%, the foam height was 7 cm, the foam retention was 3.0 mins, cleaning action was 29.72%, foam ability and foam stability was 21ml and there was no skin irritation. For F4; it is dark green in appearance with a pleasant odour. The viscosity was found to be 44 cps, pH level was 4.92, percentage of solid contents was 20.06%, the foam height was 5.6 cm, the foam retention was 4.1 mins, cleaning action was 23.50%, foam ability and foam stability was 20.5 ml and there was no skin irritation as shown in the table 5.3. Then the 4 formulations were further sent for antifungal activity in Col. Dr. Jeppiaar Research Park in Sathyabama Institute of Science & Technology. Among them the F3 shows the best physicochemical properties and maximum antifungal activity on *Candida albicans* which showed around 10mm, 19mm, 14mm and 15mm zone of inhibition in agar well of 25 μ l, 50 μ l, 75 μ l and 100 μ l concentration respectively as shown in the figure 5.15

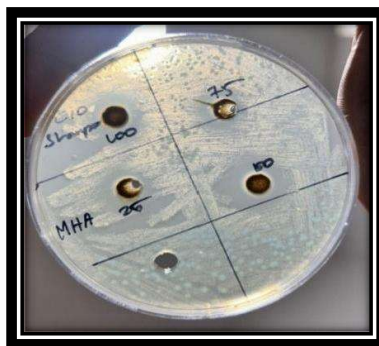


FIGURE 5.15 Zone of inhibition of *Candida albicans* in herbal shampoo.

TABLE 5.3 Evaluation parameters of formulated antifungal herbal shampoo

EVALUATION PARAMETERS	F1	F2	F3	F4
Colour	Green	Mild Green	Green	Dark Green
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Viscosity(cP)	48	50	57	44
pH	4.64	5.18	5.59	4.92
Solids contents (%)	22.11	20.32	28.26	20.06
Foam Height (cm)	8	7.5	7	5.6
Foam Retention (min)	2.3	2.8	3.0	4.1
Cleaning action (%)	19.81	24.21	29.72	23.50
Foam ability and foam stability (ml)	22	19.8	21	20.5
Skin irritation	No	No	No	No
Zone of inhibition (mm) at 50 µl concentration	15.6	16.2	19	13

ANTIFUNGAL HERBAL HANDWASH:

The physical evaluation of herbal handwash formulations F1, F2, F3 and F4 include color, odour, viscosity, pH, stability, foam height, foam retention and skin irritability were measured. For F1; it is green in appearance with a pleasant odour. The viscosity was found to be 51 cps, pH level was 6.42, the foam height was 12 cm, the foam retention was 3.4 mins, spreading coefficient was 3.5 cm and there was no skin irritation. For F2; it is green in appearance with a pleasant odour. The viscosity was found to be 54 cps, pH level was 6.89, the foam height was 8 cm, the foam retention was 3.0 mins, spreading coefficient was 4.4 cm and there was no skin irritation. For F3; it is green in appearance with a pleasant odour. The viscosity was found to be 58 cps, pH level was 7.44, the foam height was 9 cm, the foam retention was 2.4 mins, spreading coefficient was 3.1 cm and there was no skin irritation. For F4; it is green in appearance with a pleasant odour. The viscosity was found to be 49 cps, pH level was 5.82, the foam height was 7.5 cm, the foam retention was 3.8 mins, spreading coefficient was 3.9 cm and there was no skin irritation as shown in the table 5.4. Then the 4 formulations were further sent for antifungal activity in Col. Dr. Jeppiaar Research Park in Sathyabama Institute of Science & Technology. Among them the F1 shows the best physicochemical properties and maximum antifungal activity on *Candida albicans* which showed around 15mm, 16mm, 18mm and 16mm zone of inhibition in agar well of 25 μ l, 50 μ l, 75 μ l and 100 μ l concentration respectively as shown in the figure 5.16

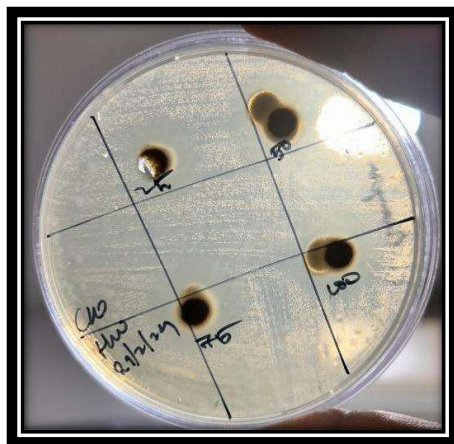


FIGURE 5.16 Zone of inhibition of *Candida albicans* in herbal handwash

TABLE 5.4 Evaluation parameters of formulated antifungal herbal handwash

EVALUATION PARAMETERS	F1	F2	F3	F4
Colour	Green	Green	Green	Green
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Viscosity(cP)	51	54	58	49
pH	6.42	6.89	7.44	5.82
Foam Height (cm)	12	8	9	7.5
Foam Retention (min)	3.4	3.0	2.4	3.8
Spreading coefficient (cm)	3.5	4.4	3.1	3.9
Skin irritation	No	No	No	No
Zone of inhibition(mm) at 75 µl concentration	18	13	14.7	12

COMPARATIVE STUDY OF PREPARED FORMULATION VS MARKETED PRODUCTS:

HERBAL SOAP Vs MARKETED PRODUCTS:

A comparative study was performed with our formulated F3 soap against two marketed products—one is herbal soap and the other is a medicated Ketoconazole soap as explained in the table 5.5. In herbal soap, the color and aroma were whitish cream with mild orange fragrance. The pH level was 5.5, the foam retention was 10.0 min, the foam height was 16.5 cm, the percentage of free alkali was 0.8%, the alcohol insoluble matter was 23.8%, the total fatty matter was 72%, the moisture content was 3.5%, the foam forming ability was 11 cm and there was no skin irritation. In medicated-Ketoconazole soap, the color and aroma were creamy white with pleasant odour. The pH level was 7.16, the foam retention was 17.12 min, the foam height was 18.26 cm, the percentage of free alkali was 1.06%, the alcohol insoluble matter was 22.5%, the total fatty matter was 71.13%, the moisture content was 9.14%, the foam forming ability was 18 cm and there was no skin irritation. Our formulated product has shown a better pH value 5.5 which is near to the reference standard pH value 5.4-5.9 of skin. It also shows a good foaming retention of 3.5 mins, foam height of 2.5 cm and foaming ability of 8 cm. Hence our formulated F3 soap has shown better results than the marketed products as shown in the figure 5.17

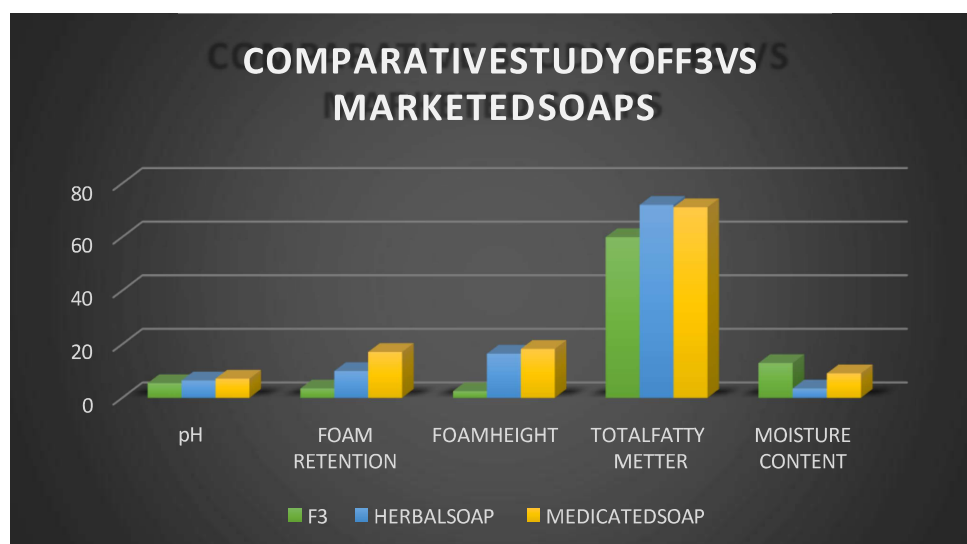


FIGURE 5.17 Graph on comparative study of F3 Vs marketed soaps

TABLE 5.5 Comparative study of F3 Vs marketed soaps

EVALUATION PARAMETERS	F3	HERBAL SOAP	MEDICATED SOAP- KETOCONAZOLE SOAP
Color	Dark green	Whitish cream	Creamy white
Odour	Pleasant	Mild orange fragrance	Pleasant
pH	5.5	6.5	7.16
Foam retention (min)	3.5	10.0	17.12
Foam height (cm)	2.5	16.5	18.26
Percentage free alkali (%)	2.3	0.8	1.06
Alcohol insoluble matter (%)	17.4	23.8	22.5
Total fatty matter (%)	60	72	71.13
Moisture content (%)	13	3.5	9.14
Foam forming ability (cm)	8	11	18
Skin irritation	No	No	No

HERBAL SHAMPOO Vs MARKETED PRODUCTS:

A comparative study was performed with our formulated F3 shampoo against two marketed products—one is herbal shampoo and the other is a medicated-ketoconazole shampoo as explained in the table 5.6. In herbal shampoo; the color and aroma were golden yellow with pleasant odour. The viscosity was found to be 50 cps, pH level was 6.4, percentage of solid contents was 24.13%, the foam height was 3.4 cm, the foam retention was 4 mins, cleaning action was 27.21%, foamability and foam stability was 20.5 ml and there was no skin irritation. In medicated-ketoconazole shampoo; the color and aroma were red/orange with pleasant odour. The viscosity was found to be 58 cps, pH level was 5.2, percentage of solid contents was 29.57%, the foam height was 6.5 cm, the foam retention was 2 mins, cleaning action was 32.61%, foamability and foam stability was 22.8 ml and there was no skin irritation. Our formulated product has shown a better pH value 5.59 which is near to the reference standard pH values slightly higher than 5.5 of scalp. It also shown a good foaming retention of 3.0 mins, foam height of 7 cm and foaming ability and foam stability of 21 ml. Hence our formulated F3 shampoo has shown better results than the marketed products as shown in the figure 5.18

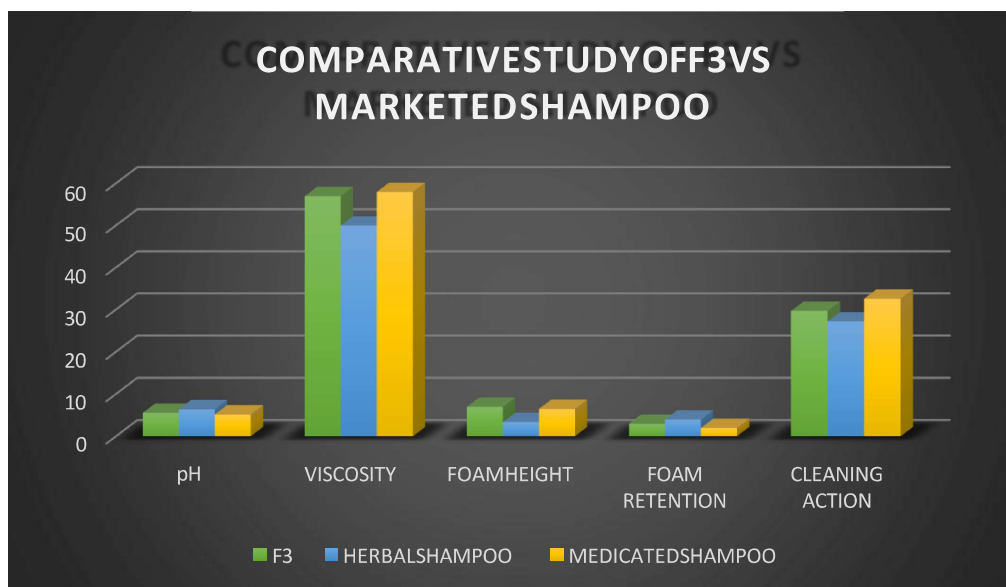


FIGURE 5.18 Graph on comparative study of F3 Vs marketed shampoo

TABLE 5.6 Comparative study of F3V marketed shampoo

EVALUATION PARAMETERS	F3	HERBAL SHAMPOO	MEDICATED- KETOCONAZOLE SHAMPOO
Colour	Green	Golden yellow	Red/Orange
Odour	Pleasant	Pleasant	Pleasant
Viscosity (cP)	57	50	58
pH	5.59	6.4	5.2
Solid contents (%)	28.26	24.13	29.57
Foam height (cm)	7	3.4	6.5
Foam retention (min)	3.0	4	2
Cleaning action (%)	29.72	27.21	32.61
Foamability and foam stability (ml)	21	20.5	22.8
Skin irritation	No	No	No

HERBALHANDWASH VsMARKETEDPRODUCTS:

A comparative study was performed with our formulated F1 handwash against two marketed products—one is marketed herbal handwash and the other is medicated handwash as explained in the table 5.7. In marketed herbal handwash, the color and aroma were brownish white with soothing odour. The viscosity was found to be 64 cps, pH level was 6.3, the foam retention was 6 min, the foam height was 11 cm, spreading coefficient was 3.7 cm and there was no skin irritation. In medicated handwash the color and aroma were creamy white with pleasant aroma. The viscosity was found to be 58 cps, pH level was 6.9, the foam retention was 4 min, the foam height was 9 cm, spreading coefficient was 4 cm and there was no skin irritation. Our formulated product has shown a better pH value 6.42 which is near to the reference standard pH value slightly higher than 6.5-6.9. It also shown a good foaming retention of 3.4 mins, foam height of 12 cm and spreading coefficient of 3.5 cm. Hence our formulated F1 handwash has shown better results than the marketed products as shown in the figure 5.19

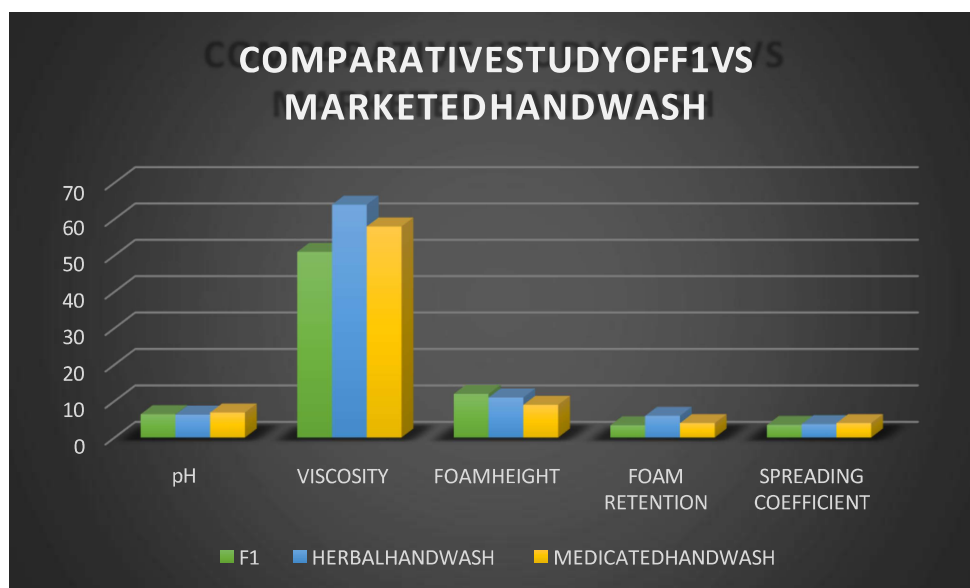


FIGURE 5.19 Graph on comparative study of F1 vs marketed handwash

TABLE 5.7 Comparative study of F1 vs marketed handwash

EVALUATION PARAMETERS	F1	MARKETED HERBAL HANDWASH	MEDICATED HANDWASH
Colour	Green	Brownishwhite	Creamywhite
Odour	Pleasant	Soothingaroma	Pleasant
Viscosity (cP)	51	64	58
pH	6.42	6.3	6.9
Foamheight (cm)	12	11	9
Foamretention (min)	3.4	6	4
Spreading coefficient(cm)	3.5	3.7	4
Skinirritation	No	No	No

DISCUSSION:

- The aim of the study was to formulate and evaluate antifungal herbal soap, shampoo, handwash using natural ingredient by agar diffusion method.
- Three formulations were formulated by the addition of extraction to a melted glycerine soap base and their organoleptic physical, moisture content, total fatty matter, pH, alcohol insoluble matter and foam height properties were assessed.
- Formulation of herbal shampoo was done by addition of extraction - active ingredient, castor oil, polyethylene glycol, glycerine, methyl paraben as (synthetic ingredient) etc... were added and their organoleptic physical and chemical characteristic properties were assessed.
- Formulation of herbal handwash was done by addition of extraction - active ingredient, glycerol, carbapol, SIs for foaming nature, methyl paraben as (synthetic ingredients) and rose oil for fragrance were added and their organoleptic physical and chemical characteristic properties were assessed.

ANIL KUMAR et al., (2013)¹⁰⁷ this study concludes that the methanolic extract of leaf and flower shows antibacterial activity and the ethanolic extract of *Sphagneticola trilobata* leaves shows the moderate anti-microbial activity against the bacterial pathogens, this study is similar to the study conducted by **A. TADDEI et al., (1999)**⁹ **AREEYASUCHANTABUD et al., (2017)**⁹ **SULEKHA AFZAL et al., (2021)**¹⁰⁸ **MUSARRATSABNAM et al., (2014)**¹⁰⁹ their study concludes that the chloroform fraction of *Sphagneticola trilobata* shows the low antibacterial and antifungal activity against the tested organisms. The above two study^{107,109} is controversial to the study conducted by **REMYA et al., (2021)**.⁷³ According to this study the ethyl acetate extract of whole plant species of *Sphagneticola trilobata* shows maximum anti-microbial activity, The methanolic extract of plant part shows moderate antimicrobial activity, Petroleum ether extract shows the least antimicrobial activity compared with ethyl acetate & methanolic extracts. whereas **C. KARTHIKA et al., (2018)**⁸⁷ this study concludes that the phytochemical evaluation of leaves extract *Sphagneticola trilobata* was done for the presence of alkaloids, flavonoids, Tannins, saponins, Phenol, Carbohydrates, Glycosides, etc.. This study is similar to the study conducted by **CIENAARURAL et al., (2019)**¹¹⁰,

SUSHAMARAJRV *et al.*, (2019)¹¹¹ and **SAIPRASANNAK** *et al.*, (2019)¹¹² this study is controversial to the study conducted by **SANGEETA S SUTAR** *et al.*, (2020)⁸⁰ according to this study starch, proteins, & alkaloids are present in roots; tannins, saponins and fats are present in stem and leaves, absent in roots and glycosides present in leaf and absent in stem & root. Due to the presence of these active constituents in the ethyl extract of *Sphagneticola trilobata* shows antimicrobial activity. **ZHE WANG** *et al.*, (2021)¹¹³ this study concludes that the extraction of *Sphagneticola trilobata* shows anti-inflammatory activity but our study proved that the *Sphagneticola trilobata* shows more therapeutic benefits like antimicrobial, antifungal activity etc...and has more benefits in other treatments, effectiveness, less side effects and less expensive. The Results of methanolic extract of *Sphagneticola trilobata* leaves shows moderate activity which conducted by **VIVIMARDINAL** *et al.*, (2020)¹¹⁴ whereas this study is controversial to the study conducted by **SYAFRUDDIN ILYAS** *et al.*, (2021)¹¹⁵ shows that extract of *Sphagneticola trilobata* contains potent antimicrobial activity using ethyl acetate. **SA NIRMAL** *et al.*, (2005)^[78] this study concludes that antibacterial activity of the essential oil was evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* this is similar to **RS VERMA** *et al.*, (2014)⁷⁶ **GOVINDAPPA** *et al.*, (2011)¹¹⁴ concludes that extracts of *S. trilobata* showed more potent activity against antimicrobial activity may be due to strong occurrence of polyphenolic compounds such as flavonoids, Tannins, terpenoids, phenol, saponin etc.. This result indicates the possibility of using whole plant of *Sphagneticola trilobata* to prove its antimicrobial activity with its secondary metabolites.

DICUSSION ON ANTIFUNGAL HERBAL SOAP:

RAKESH.K *et al.*, (2011)⁹⁰ this study concludes that the soap was prepared by using herbal ingredients like coconut oil, neem oil, menthal oil, rose petals and different extract was incorporated for basic saponification reaction whereas our study concludes that the herbal soap was prepared only by using drug extract, glycerine soap base and rose oil for fragrance which shows antimicrobial activity naturally by the extract itself.

DISCUSSION ON ANTIFUNGAL HERBAL SHAMPOO:

Vivek Sharma et al., (2019)¹¹⁶ this study explains the evaluation parameter of Herbal Shampoo was tested for physical appearance, Ph, odour, Solid contents (%), Foam ability and Foam stability whereas our study concludes that herbal shampoo was tested for physical appearance, Ph, Rheological evaluation, odour foamability and Foam stability, Dirty dispersion, cleaning action, viscosity to prove consistency.

DISCUSSION ON ANTIFUNGAL HERBAL HANDWASH:

Komsaltikariya et al., (2013)¹¹⁷ this study explains that formulation of herbal hand wash uses neem, sls, aloe vera juice, carbacol, glycerine, rose oil, methyl paraben and distilled water but our study we used acacia as gelling agent instead of carbacol which shows stable physical parameter with good cleaning agent.

CHAPTER – 6
SUMMARYANDCONCLUSION

CHAPTER – 6

SUMMARY AND CONCLUSION

SUMMARY:

Throughout this study, we found initially, the identification of phytochemical constituents, presents and absence in various extracts of whole plant *S. trilobata*. The distilled water, Ethanol and Ethyl Acetate extracts are involved in the Phytochemical analysis and all three showed the presence of Alkaloids, Flavonoids, Saponins, Steroids, Tanins, Proteins and Phenol. The Anti-microbial agar-well diffusion assay was performed further and the result encloses the presence of gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. coli*) along with the fungi *Candida Albicans* in the Ethyl Acetate extract. Whereas, the distilled water and Ethanol extract showed negative zone of Inhibition against Anti-Microbial activity.

Thus, the Ethyl Acetate extract is involved for the formulation and evaluation of herbal cosmetic products like soap, shampoo and handwash. We conducted a series of formulation for all the cosmetic products to optimize the better formulation among it and undergone for the evaluation parameter according to the products requirements. All three cosmetic products are subjected to analyse the Anti-fungal activity against *Candida Albicans*, they proved positive activity along with an effective zone of inhibition. This study induct the significant of Anti-fungal activity highlighting the potential of whole plant *S. trilobata* as a nature source for the formulation of herbal cosmetic products.

CONCLUSION:

Based on the formulation and evaluation of herbal cosmetics products using *Sphageticola trilobata* ethyl acetate extract, the study concludes that this extract shows potential for use in the development of natural and effective cosmetic products. Further research and testing are recommended to explore its full range of benefits and ensure its safety for long-term use. The study highlights the importance of harnessing the power of natural ingredients in the cosmetics industry.

STRENGTH OF THE STUDY:

Ethyl acetate extract of *Sphageticola trilobata* confirms the activity of Anti-oxidant in many studies but our study confirms the activity of Anti-microbial against

gram positive and gram-negative bacteria. Our study has proved the extract of whole plant of *Sphagneticola trilobata* can be employed in the formulation of herbal preparation, which has not been formulated before. Among the various species of trilobata, our study concentrate on a particular species known as *Spageticola trilobata*, which is studied to have rich phytochemical and pharmacological activity than the other trilobata species.

LIMITATIONS:

Although most of the studies have worked on the methanolic extract of *s.trilobata* to find out the anti-microbial activity, methanolic extract is not the choice of chemical for extraction in our study due to the use of methanol is prohibited. Though it has the highest Anti-microbial activity. In place of methanol, we chose ethyl acetate extract due to their potent activity among the other chemical extracts. Like petroleum ether, chloroform etc...

FUTURE SCOPE:

The future scope of the study on the formulation and evaluation of *Sphagaticola trilobata* ethyl acetate extract is quite promising. Some potential areas for further exploration and development include:

1. **Optimization of Formulations:** Researchers can continue to fine-tune the formulation of cosmetics using *Sphagaticola trilobata* extract to maximize its efficacy and stability.
2. **Ingredient Synergy:** Investigating the compatibility and synergistic effects of *Sphagaticola trilobata* extract with other natural ingredients could lead to the development of more potent and multifunctional cosmetic products.
3. **Clinical Studies:** Conducting clinical studies to evaluate the long-term safety and efficacy of cosmetics formulated with *Sphagaticola trilobata* extract can provide valuable data for regulatory approval and consumer confidence.
4. **Product Diversification:** Exploring the use of *Sphagaticola trilobata* extract in various cosmetic products beyond skincare, such as hair care, body care, and makeup, can expand its potential applications and market reach.

5. Sustainability and Ethical Sourcing: Further research can focus on sustainable cultivation methods, ethical sourcing practices, and the environmental impact of utilizing *Sphagaticola trilobata* extract in cosmetics.

6. Anti-fungal property: By conducting further research, scientists can determine the effectiveness of the extract against different types of fungi and compare it to existing anti-fungal agents. This could pave the way for the development of natural and effective treatments for fungal infections in various applications, including cosmetics. Exciting possibilities lie ahead in harnessing the anti-fungal properties of *Sphagaticola trilobata* ethyl acetate extract.

7. To perform short-time and long-time stability test

By exploring these avenues, the study can contribute to the development of innovative, effective, and environmentally friendly herbal cosmetics products.

ANNEXURE



சித்தமருத்துவமைய ஆராய்ச்சிநிலையம்
மத்தியசித்தமருத்துவ ஆராய்ச்சிக்குழுவும், ஆயுஷ் அமைச்சகம், இந்தியா (அரசு)

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AUTHENTICATION CERTIFICATE FOR 663.01122301

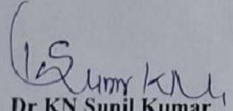
Certified that the sample submitted by S Harihara Suthan, K Kamesh, M Anishiya, D Kesavardhini, R Sujipriya, B.Pharm Final year, Sathyabama Institute of Science and Technology, Rajiv Gandhi Salai, Chennai - 600 119 was identified as:

Code	Botanical Name	Part
S01122301T	<i>Sphagneticola trilobata</i> (L.) Pruski	Whole plant

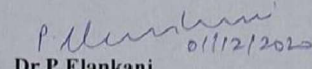


S01122301T

*WFO (2023): *Sphagneticola trilobata* (L.) Pruski. Published on the Internet:
<http://www.worldfloraonline.org/taxon/wfo-0000101479>; Accessed on 01 Dec 2023


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