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HERBAL SYNERGY IN CLEANSING: *ALOE VERA* AND *LEUCAS ASPERA* -BASED SOAP EVALUATION

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ABSTRACT

The aim of the project is to formulate and evaluate the different parameters of the poly herbal soap formulation which was performed in the lab. Which contains mainly Leucas aspera leaves extract, Aloe barbadensis gel extract in it. The poly herbal soap was subjected to various evaluation parameters like colour, odour, pH, foam height, washability, foamability. As F3 shows good spreadability, washability and the foamability and which is required for ideal poly herbal soap. The antimicrobial test proved that F3 has better antimicrobial property compared to F1 and F2. So, compared to F1 and F2 formulations, the F3 shows the best in all parameters, so, was more acceptable than other two formulations. So, this shows that herbal formulation has benefits over the synthetic formulation which contains harmful chemicals that can harm our skin. The herbal formulations are less toxic or have less or no side effects. They are safe and eco-friendly and suitable for all skin types.

KEYWORDS: Herbal soap, Antimicrobial activity, irritancy, Spreadability.

INTRODUCTION

The skin is the biggest organ of the body, accounting for approximately 15% of the whole adult body weight. It plays many crucial functions, including protection against external physical, chemical, and biologic assailants, in addition to prevention of excess water loss from the body and a position in thermoregulation. The skin protects us from microbes and the elements, facilitates adjust body temperature, and allows the sensations of touch, heat, and cold. The skin is continuous, with the mucous membranes lining the body's surface.[1]

The integumentary system is shaped by the skin and its derivative structures. The skin consists of three layers: the epidermis, the dermis, and subcutaneous tissue. The outer most level, the epidermis, includes a specific constellation of cells known as keratinocytes, which function to synthesize keratin, a long, threadlike protein with a defensive role. The middle layer, the dermis, is basically made up of the fibrillar structural protein known as collagen. The dermis lies on the subcutaneous tissue, or panniculus, which includes small lobes of fat cells known as lipocytes. The thickness of these layers varies considerably, relying on the geographic region on the anatomy of the body. The eyelid, for example, has the thinnest layer of the epidermis, measuring much less than 0.1 mm, whereas the palms and soles of the feet have the thickest epidermal layer, measuring about 1.5 mm. The dermis is thickest on the back, where it is 30-40 times as thick as the overlying epidermis.[1]

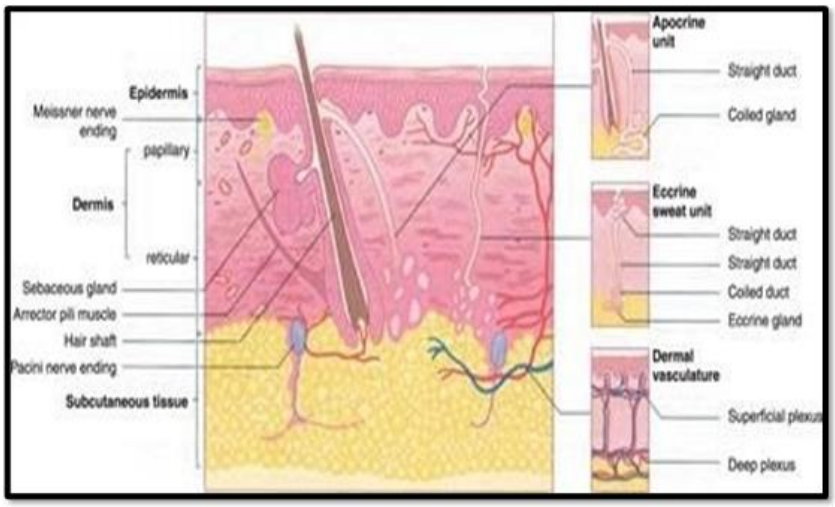

Figure 1.1 Structure of the Skin.

SKIN ANATOMY

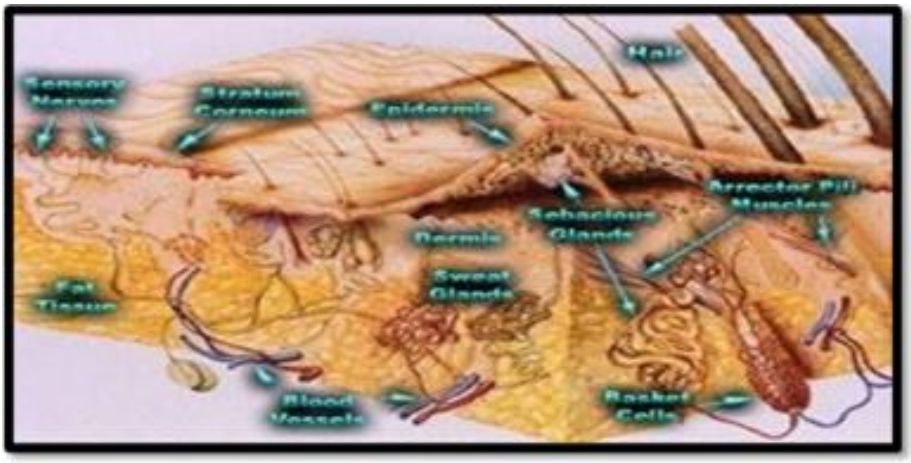

Figure 1.2: Anatomy of the Skin.

A fundamental understanding of skin anatomy is crucial when explaining the process of skin biopsy. Each element of the skin plays a role in its daily function; therefore, every element is a source of important information that can be captured and assessed with a skin biopsy.[2]

- **Hair** - Hair serves a defensive role in the skin. On most locations of the body, hair offers a defensive covering, which regenerates on a regular basis. In some places, hair serves as a filter (such as in the nose and ears), a moisture and heat retention mechanism (such as the armpits and genital region), and in the middle ear it serves as a mechanism for regulating balance. Each hair follicle (in the hairy parts of the skin) is connected to a muscle, the arrector pili (see Arrector Pili for more information).[2]
- **Stratum Corneum** - This is the dead skin layer that is seen when you look at your skin. It functions to protect the living cells below by providing a hard barrier between the outside world and the delicate cells inside. The stratum corneum is beneficial for diagnosis because in some conditions the stratum corneum will become thinner than normal.
- **Epidermis** - The epidermis is the following layer under the stratum corneum. Its function is to guard the body. It produces cells that will ultimately become stratum corneum cells. It contains sensory nerves especially small diameter sensitive temperature fibers. It is these sensory nerves that are helpful when evaluating a skin biopsy.
- **Sensory Nerves** - These are the nerves that innervate the epidermis. These nerves are the subject of evaluation when examining a skin biopsy after it has been immune stained. The sensory nerves in the epidermis serve to sense and transmit heat, pain, and other noxious sensations. When these nerves are not functioning properly, they can produce sensations such as numbness, pins-and-needles, pain, tingling, or burning. When evaluated, traits of the nerve such as total number, contiguity, diameter, branching, swelling, and overall fitness are taken into consideration.
- **Dermis** - The dermis is the following layer under the epidermis. The dermis contains all of the other

sub-epidermal structures mentioned below. Dermis is characterised by loose, ribbon-like cells that hold dermal structures in place and serves to contain fluids.

- **Arrector Pili Muscle** - This is a tiny muscle that attaches to the base of a hair follicle at one end and to dermal tissue on the other end. In order to generate heat when the body is cold, the arrector pili muscles contract all at once, causing the hair to "rise up straight" on the skin. The arrector pili muscle is a source of information when evaluating a skin biopsy since it is well-innervated with autonomic nerves that control when the muscle contracts. These autonomic nerves are also visible when the skin biopsy is immune stained.
- **Sebaceous Glands** - These structures are related closely with hair follicles because they produce an oily substance that coats and protects the hair shaft from becoming brittle.
- **Sweat Glands** - These glands produce moisture (sweat) which is secreted through tiny ducts to the floor of the skin (stratum corneum). The moisture serves as a cooling agent by making the floor of the skin moist. This moisture then evaporates and lowers the temperature of the skin.
- **Basket Cells** - These structures surround the base of hair follicles and serve as stress sensors. They are a source of treasured information when assessing general nerve health and condition.
- **Blood Vessels** - These structures carry critical nutrients and oxygen-rich blood to the cells that make up the layers of skin and then carry away waste products. Often, the blood vessels are in near proximity to collections of nerve bundles in the dermal and sub-dermal layers.[2]

SKIN PHYSIOLOGY

The physiological features of the skin include.

- **Protection** against microorganisms, dehydration, ultraviolet light, and mechanical damage; the skin is the primary physical barrier that the human body has towards the external environment.
- **Sensation** of pain, temperature, touch, and deep stress starts with the skin.
- **Mobility:** The skin permits easy movement of the body.
- **Endocrine activity:** The skin initiates the biochemical processes involved in Vitamin D production that is vital for calcium absorption and normal bone metabolism.
- **Exocrine activity:** This occurs by the release of water, urea, and ammonia. Skin secretes products like sebum, sweat, and pheromones and exerts vital immunologic features by secreting bioactive substances including cytokines.
- **Immunity** development against pathogens.
- **Regulation of Temperature**. Skin participates in thermal regulation by preserving or releasing heat and allows maintain the body's water and homeostatic balance.
- **Perspiration:** In investigating human perspiration or sweating, we may also measure the levels of lactate and urea that are the major sweat constituents. The concentration profiles of lactate and urea display higher amounts in the skin surface and drop hastily below the surface. In the neonate, the sweat glands have not completely formed andthe secretory coils of the glandular segment and the sweating response to external stimuli are limited.
- **Skin hydration:** Capacitance values correspond to stratum corneum hydration, which affects barrier mechanical properties and percutaneous absorption. At birth, the skin surface is rougher and dryer as compared with older children. During the first 30 days of life, skin smoothing is correlated to an increase in skin hydration. During the following 3 months, the hydration of the stratum corneum will increase and exceeds the hydration level found in adults. The functional maturation of sweat glands can be the fundamental mechanism related to the increase in skin hydration after birth.
- **Skin pH:** pH is defined as the negative logarithm of the activity of hydrogen ions in an aqueous solution, used to express acidity and alkalinity on a scale of 0–14. Normal values of pH in intact adult skin are acidic because of the presence of the acid mantle, at the same time as the interstitial fluid is characterised through neutral values. Infant skin pH levels are higher than those of adult skin, that is usually characterised by a pH value among 5 and 5.5. New borns have alkaline skin surfaces, starting from 6.34 to 7.5, relying on the anatomical site.[3]

TREATMENT

Skin diseases are numerous and an often-occurring health problem affecting all ages from the neonates to the aged and cause damage in number of ways. Maintaining healthy skin is critical fora healthy body. Many people may develop skin diseases that affect the skin, including cancer, herpes and cellulitis. Some wild plants and their parts are frequently used to treat these diseases. The use of plants is as old as the mankind. Natural remedy is cheap and claimed to be safe.[4]

HERBAL DRUGS FOR SKIN DISEASE

Natural drugs from the plants are gaining popularity because of several benefits such as often having fewer side-effects, higher patient tolerance, being relatively less expensive and acceptable due to a long history of use. Besides herbal medicines offer rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. For these reasons several plants have been investigated for treatment of skin diseases starting from itching to skin cancer. So far more than 31 plants have been reported to be effective in various skin diseases during the-past 17 years, Examples of few herbal medicinal plants are stated below.[4]

Curcuma longa (Common name: Turmeric; Family:

Zingiberaceae).-A study conducted on male Swiss albino mice in whom skin cancer was induced by topical application of DMBA, revealed a significant reduction in number of tumors per mouse in the group receiving 1% curcumin obtained from rhizomes of C. longa.

Azadirachta indica (Common name: Neem; Family: Meliaceae)-Leaf extract is applied externally on boils and blisters. It additionally shows anti-acne activity when applied to face or used in cosmetic preparations.

Aloe barbadensis (Common name: Aloe vera; Family: Asphodelaceae)-Aloe vera has shown excellent results in skin diseases and it is frequently taken as health drink. It is also found effective in treating wrinkles, stretch marks and pigmentations. It also seems to be able to pace wound healing by enhancing blood circulation through the area and preventing cell death around a wound.

Achyranthes aspera (Common name: Prickly chaff flower, Devil's horsewhip; Family: Amaranthaceae)-Traditionally, the plant is used in boils, scabies and eruptions of skin and other skin diseases. Allium cepa (Common name: Onion; Family: Liliacea). A study undertaken in sufferers with seborrheic keratoses to evaluate the ability of onion extract gel to enhance the appearance of scars following excision, has shown that this extract gel improved scar softness, redness, texture and global appearance at the excision site.[4]

Skin care preparations

The skin care preparations have grown phenomenally over the years. People utilize range of skin care preparations, from mouth wash, from lipsticks to complexion creams to foot powders and so on, in the hope of developing a charming personality, protecting their bodies and avoiding bad smell. Skincare preparations are described as substances that are intended to be rubbed, poured, sprinkled and sprayed-on or, introduced into or otherwise applied to human body or any part of body, for cleansing, beautifying, promoting attractiveness or altering the appearance of skin.[5]

Herbal Soap

Herbal soaps can be defined as fatty acids in combination with alkali salts being derived from vegetable or plant origin containing natural fragrances or organic ingredients. The method of preparation is by two processes - hot process and cold process which involves the presence of base such as potassium hydroxide and sodium hydroxide along with the fatty acids to form soap. Cold process is usually preferable process by the artisans. The quality of the soaps is dependent on various factors such as type of alkali used, its hardness, foam height, solubility etc.[6]

Figure 1.3 Poly herbal marketed soaps.

ADVANTAGES OF HERBAL SOAP

- Lesser Side effects
- Better safety and efficacy
- Easily available
- Better compatibility with additives
- Potent therapeutic effect
- Cost-friendly
- Greater are for selection
- No requirement of animal testing
- Better compatibility with all types of skin.[6]

LEUCUS ASPERA

- Common name: Thumbe
- Family: Lamiaceae

Leucas aspera is a plant species within the genus Leucas and the family Lamiaceae. Although the species has many different common names depending on the region in which it is located, it is most commonly known as Thumbai or Thumba. Found throughout India, it is known for its various uses in the fields of medicine and agriculture.[9]

The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity. Further, studies reveal the presence of various phytochemical constituents mainly triterpenoids, oleanolic acid, ursolic acid and bsitosterol, nicotine, sterols, glucoside, diterpenes, phenolic compounds (4-(24-hydroxy-1- oxo-5-n- propyltetracosanyl)-phenol). These studies reveal that L. aspera is a source of medicinally active compounds and have various pharmacological effects; hence, this drug encourage

finding its new therapeutic uses. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.[10]

Figure 2.1 Leucas aspera.

ALOE VERA (ALOE BARBADENSIS)

- Common name: Aloe vera
- Family: Asphodelaceae

Figure 2.2 Aloe barbadensis leaf.

The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. It's full of vitamins, minerals, and anti-inflammatory compounds that provide healing relief from irritation. Aloe vera soothes sunburns and skin injuries by increasing collagen synthesis and cross-linking. This helps to reduce any resulting scar tissue and speeds up wound healing. Aloe also contains compounds called aloin and anthraquinone, which have anti-inflammatory and antioxidant benefits that can help alleviate pain and promote healing. Since aloe vera contains humectants (substances that attract water from the air or from deeper in the skin), it's thought to be especially beneficial for dry skin types. It also has cohesive effects by sticking together flaking epidermal skin cells, resulting in softer skin and improved skin integrity. There are two compounds in aloe that may fade dark spots and stretch marks: aloesin and aloin. Aloe vera contains naturally occurring salicylic acid, urea nitrogen, cinnamic acid, phenols and sulfur—all of which inhibit the growth of fungi, bacteria and viruses. Because aloe vera contains vitamins C and E, it stands to reason it may help prevent the formation of free radicals, which are the molecules that cause cell damage.[11]

METHODOLOGY MATERIALS

Equipment's and apparatus: Soxhlet extractor, beaker, China dish, separating funnel, pipette, glass rod, heating

mantle, hot water bath, digital pH meter, Petri dish.
Ingredients: Leucas aspera, Aloe vera, lemon grass oil, glycerine soap base.

METHODS

EXTRACTION OF LEUCAS ASPERA LEAVES

The Leucas aspera leaves were extracted using Soxhlet extraction method. The leaves of leucas aspera were dried under the sunlight and 36.8g of the leaves were powdered and weighed. To 500 ml beaker the weighed leucas aspera leaves were added and 400 ml of ethanol was poured and placed in Soxhlet apparatus and extraction was conducted. The product obtained was kept in water bath for evaporation and the extraction product was obtained.[17]

Figure 4.1 Dry powder of Leucas aspera leaves.

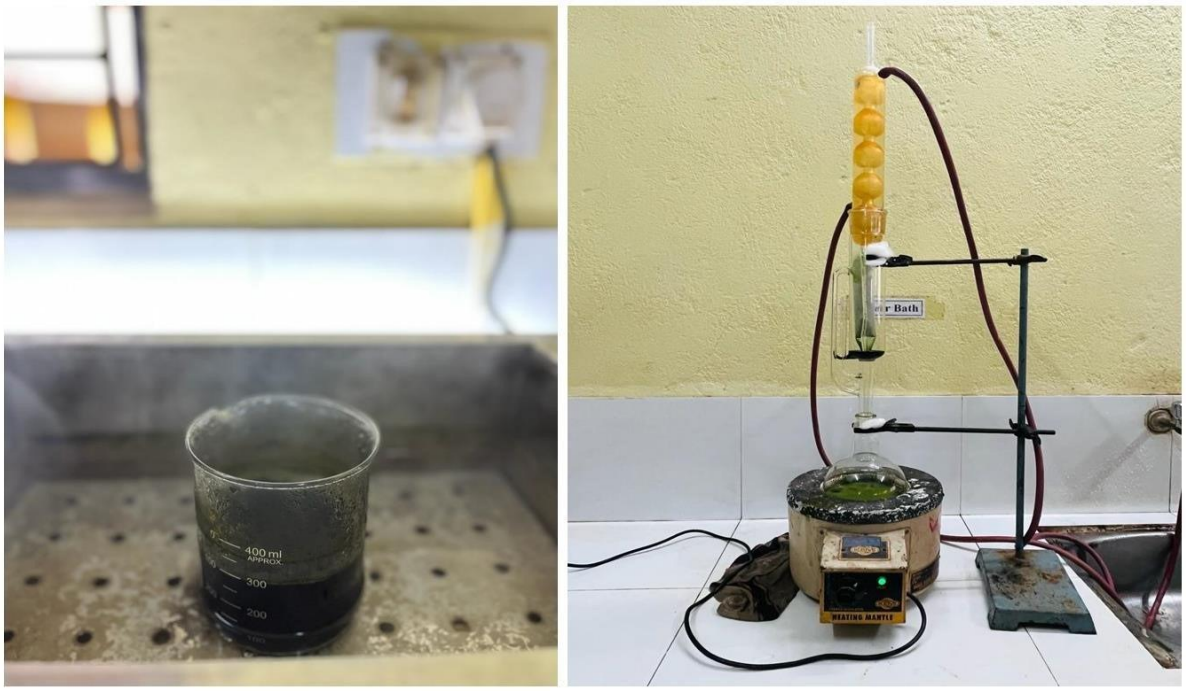

Figure 4.2 Crude extract of Leucas aspera leaves and Soxhlet extractor.

PHYTOCHEMICAL SCREENING OF LEUCAS ASPERA LEAF EXTRACT[18]

1. **Mayer's test for alkaloids** -1 or 2 drops of Mayer's reagent was added to 2 ml of crude extract.
2. **Salkowski's test for terpenoids** - 2 ml of crude extract was shaken in 1 ml of $CHCl_3$. Then add few drops of conc.H_2SO_4 solution along the side of test tube.
3. **$FeCl_3$ test for tannins** - 50 mg of crude extract was dissolved in 5 ml distilled water, followed by the addition of a few drops of 5% $FeCl_3$.
4. **Alkali test for flavonoids** - Few drops of 5% NaOH solution were added to 1 ml of filtered stock solution (100 ml of extract dissolved in 10 ml of methanol),

which produced a deep – yellow color. The color was lost in the presence of dilute HCl.

5. **Saponin test -** Foam test 1 ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes.

Figure 4.3 Chemical test for phytochemicals.

EXTRACTION OF ALOE VERA GEL

The aloe vera leaves were collected and using a knife they were cut and the outer skin was removed and the inner flesh was blended to obtain the gel.

Tab. No. 4.1: Formulation table for poly herbal soap.

S.no.	Name of ingredients	Property
1.	Leucas aspera	Anti-microbial, antioxidant
2.	Aloe vera	Anti-inflammatory, Antioxidant
3.	Lemon grass oil	Perfuming agent
4.	Glycerin soap base	Soap base

PROCEDURE FOR PREPARATION OF HERBAL SOAP

- Solidified glycerine soap base was weighed and broken down into smaller pieces.
- It was transferred into a glass beaker and then heated in a water bath to melt the soap base and stirred continuously with a glass rod.
- Further the required quantity of leucas aspera leaf extract and aloe vera gel was added to the base after the soap base was liquefied.
- The mixture was stirred continuously to avoid lumps.
- There was continuous agitation with a glass rod for 15 minutes until the molten mixture became homogeneous.
- The homogenous mixture was removed from the water bath, and lemon grass oil was added and stirred slowly.
- The semisolid mixture was poured into a mould and allowed to solidify.
- The soap was allowed to solidify at room temperature until set and kept under physical observation for any characteristic changes.[19]

Tab. No. 4.2: List of formulation.

SL.No.	Name of ingredients	F1	F2	F3
1.	Leucas aspera	1.5g	2g	2.5g
2.	Aloe vera	0.5g	1g	1.5g
3.	Lemon grass oil	5 drops	3 drops	4 drops
5.	Glycerin soap base	100g	100g	100g

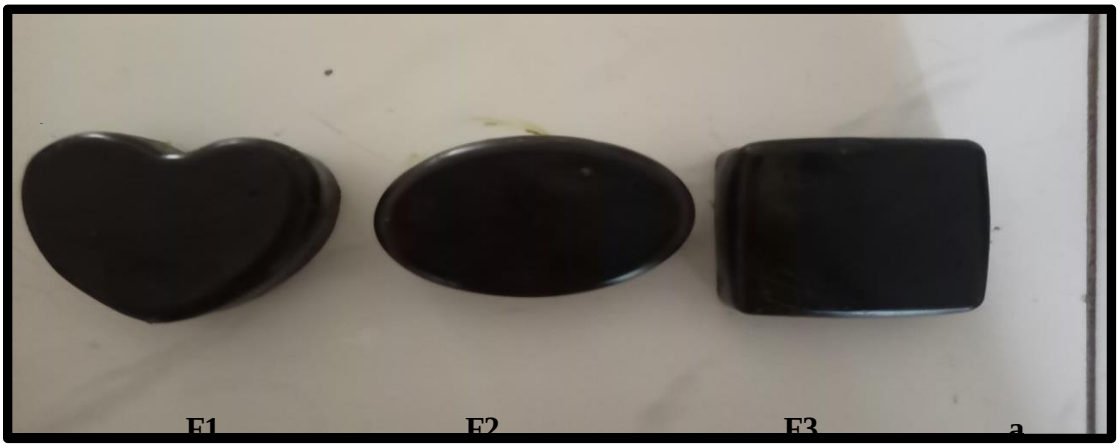

Figure 4.4 Prepared poly herbal soaps.

EVALUATION PARAMETERS

The prepared herbal soap formulation was evaluated for following parameters

1. **Colour:** The colour of the poly herbal soap was evaluated visually.
2. **Odour:** The formulation was evaluated for its odour by smelling it.
3. **pH Parameter:** pH of formulated herbal soap was determined using pH paper.

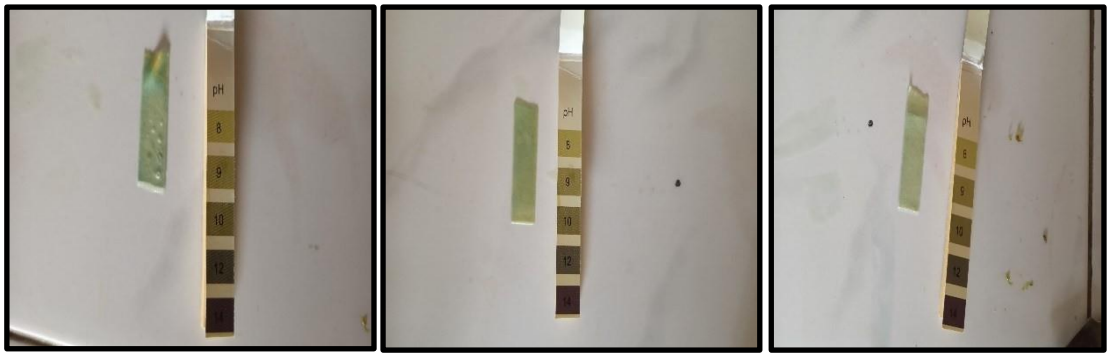

Figure 4.5 Determination of pH of poly herbal soaps.

Foam Height

- 0.5g of sample of soap was dispersed in 25 ml of distilled water.
- Then it is transferred into 100 ml measuring cylinder and the volume was made up to 50 ml with water.
- 25 strokes were given and allowed to stand till aqueous volume measured up to 50 ml and the foam height above the aqueous volume was measured.

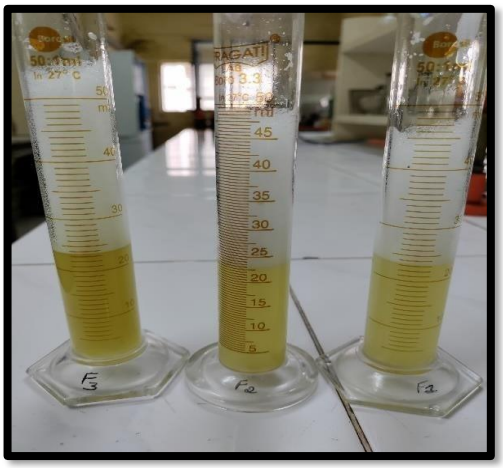

Figure 4.6 Determination of foam height of poly herbal soaps.

Washability

- Formulation was applied on the skin and then ease and extent of washing with water was checked manually.

Figure 4.7 Determination of washability test for poly herbal soaps.

Foamability

- Small amount of prepared soap was taken in a beaker containing water. Initial volume was noted, beaker was shaken for 10 times and the final volume was noted.

Figure 4.8 Determination of foamability poly herbal soaps.

Antimicrobial test

- Microorganisms used: *Staphylococcus aureus and Escherichia coli.*
- Preparation and dilution of soap sample extracts: With the help of sterile sharp knife soaps were scrapped at one side. 250 mg of each soap sample was weighed and dissolved in 1ml of sterile distilled water.
- Disc diffusion assay: Agar disc diffusion method was used to detect antimicrobial assay. The standardised 0.1 ml saline suspensions of test organisms were inoculated on the surface of the sterile Muller-Hinton agar plate. Sterile filter paper disc prepared from different concentrations of the various soap samples were aseptically transferred directly into the surface of the plate with the help of sterile forceps. All the plates were incubated at 37°C for 24 - 48 hours and then were examined for zone of inhibition around the disc. The zone of inhibition was determined by measuring the diameter in millimetre of zone to which the soap inhibited the growth of organism.

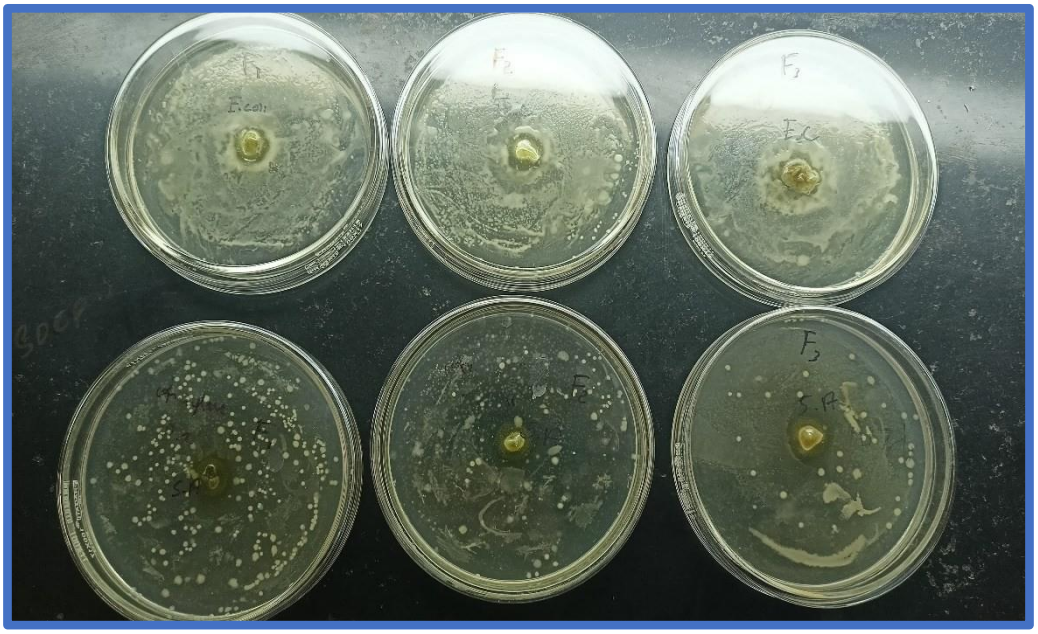

Figure 4.9 Zone of inhibition for F1, F2 and F3 formulations.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING OF LEUCAS ASPERA LEAF EXTRACT[18]

Tab. No. 5.1: Chemical test for phytochemicals.

SL No.	Test	Observation	Inference
1.	Mayer's test for alkaloids	White creamy precipitate	Alkaloids are present.
2.	Salkowski's test for terpenoids	Development of red – brown color at the interface.	Terpenoids are present.
3.	$FeCl_3$ test for tannins	Development of bluish – black color.	Tannins are present.
4.	Alkali test for flavonoids	Presence of deep – yellow color. The color was lost on addition of HCl.	Presence of flavonoids. Flavonoids are confirmed.
5.	Saponin Test :	A layer of foam was observed	Saponin are present in the sample

1. EVALUATION OF COLOUR

Tab. No. 5.2: Colour of F1, F2 and F3 formulations.

Parameter	Sample	Observation
Colour	F1	Dark green
Colour	F2	Dark green
Colour	F3	Dark green

2. EVALUATION OF ODOUR

The odour of the 3 samples that is F1, F2, F3 was found to be pleasantly citrus.

3. EVALUATION OF PH

Tab. No. 5.3: pH of F1, F2 and F3 formulations.

Parameter	Sample	Observation
pH	F1	8
pH	F2	8.2
pH	F3	7.5

4. EVALUATION OF FOAM HEIGHT

Tab. No. 5.4: Foam height of F1, F2 and F3 formulations.

Parameter	Sample	Observation
Foam height	F1	6 cm
Foam height	F2	7 cm
Foam height	F3	8.2 cm

5. EVALUATION OF WASHABILITY

Tab. No. 5.5: Washability of F1, F2 and F3 formulations.

Parameter	Sample	Observation
Washability	F1	Requires time for washing
Washability	F2	Requires time for washing
Washability	F3	Easily washable compared to F1 and F2

6. EVALUATION OF FOAMABILITY

a. The small amount of the samples was taken from F1, F2, F3 in a different beaker containing water.
b. The 3 samples showed the appearance of the foam in the different beakers.
c. The formulation F3 showed better foaming property compared to F1 and F2.

7. EVALUATION OF ANTIMICROBIAL ACTIVITY

Tab. No. 5.7: Zone of inhibition of F1, F2 and F3 formulations.

SL. No	Formulation code	Zone of inhibition (mm)	
		Microorganisms	
		Staphylococcus aureus	***Escherichia coli.***
1.	F1	21	20
2.	F2	27	15
3.	F3	34	19

CONCLUSION

- The aim of the project is to formulate and evaluate the different parameters of the poly herbal soap formulation which was performed in the lab.
- Which contains mainly Leucas aspera leaves extract, Aloe barbadensis gel extract in it.
- The poly herbal soap was subjected to various evaluation parameters like colour, odour, pH, foam height, washability, foamability.
- As F3 shows good spreadability, washability and the foamability and which is required for ideal poly herbal soap.
- The antimicrobial test proved that F3 has better antimicrobial property compared to F1 and F2.
- So, compared to F1 and F2 formulations, the F3 shows the best in all parameters, so, was more acceptable than other two formulations.
- So, this shows that herbal formulation has benefits over the synthetic formulation which contains harmful chemicals that can harm our skin.
- The herbal formulations are less toxic or have less or no side effects.
- They are safe and eco-friendly and suitable for all skin types.

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