

PREPARATION OF MEDICATED SOAP WITH ENHANCED ANTI-BACTERIAL PROPERTY AGAINST *STAPHYLOCOCCUS AUREUS*

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science degree in Microbiology

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY-1 UNIVERSITY BY UGC

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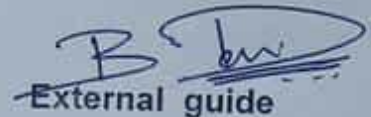
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **S.SOWMIYA (41300044)** and **E. SUBHA SHREE (41300047)** who carried out the project "PREPARATION OF MEDICATED SOAP WITH ENHANCED ANTI-BACTERIAL PROPERTY AGAINST *STAPHYLOCOCCUS AUREUS*" under our supervision from January 2024 to April 2024.



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ABSTRACT

In day-to-day life various microbes are deposited on the surface of the skin from the dust present in external environment. When a proper hygiene is not practiced, these microbes replicate, produce toxic substances and eventually cause disease. Frequently skin infections are caused by staphylococcus aureus gram positive bacteria. There are some medicated soaps which is used to kill or inhibit the growth of microbes which causes skin infections in human. These commercially available medicated soaps contain some chemicals which is not suitable for sensitive skin, which cause itching, rashes, inflammations and dryness. For avoiding such problems handmade herbal soaps which are made up of natural ingredients can be used. The aim of the present research work is to compare the efficacy of various branded soaps and handmade soap against skin infecting human pathogenic bacteria such as Staphylococcus aureus. 15 soap samples were prepared with different concentration, 250mg/ml soap concentration were subjected to staphylococcus aureus stain, using agar disk diffusion method to know how sensitive the organisms are to the soap concentrations. The Indian culture is full of traditional herbal medicines which are used in daily life by each and every Indian. The ayurveda system of medicine has cured all diseases in healthy way. Natural antibacterial soap is a type of medicated soap which is used to get rid from various bacterial infections by utilizing traditional herbal medicines such as herbal oil or plant extract. As compared to the modern medicinal techniques, the herbal soaps formulations have less side effects and wider activity of action. Hence, they are getting more popular among people now a days. These handmade herbal soaps are good for all types of skin.

Keywords: Antibacterial soap, natural ingredient, plant extract, *Staphylococcus aureus*, wide spectra of activity.

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LIST OF ABBREVIATIONS

<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>P. aeruginosa</i>	<i>Pseudomonas</i>
<i>A. indica</i>	<i>Acalypha indica</i>
<i>A. aspera</i>	<i>Achyranthes aspera</i>
<i>A. indica</i>	<i>Azadirachta indica</i>
<i>S. auriculata</i>	<i>Senna auriculata</i>
<i>C. aromatic</i>	<i>Curcuma aromatic</i>
TFM	Total Fatty Matter
BBD	Box Behnken Design
MHA	Mueller Hinton Agar
NaCl	Sodium Chloride
EDTA	Ethylenediaminetetraacetic acid
SLS	Sodium Lauryl Sulphate
EWG	Environmental Working Group
FDA	Food and Drug Administration

CHAPTER 1

INTRODUCTION

Soap and other cleaning agents have been around for quite a long time. For generations, handwashing with soap and water has been considered a measure of personal hygiene. Bacteria are very diverse and are present in water, soil, sewage and on human body. In 1961 the U.S public health service recommendation directed that to personally wash their hands in soap and water for 1 to 2 minutes before and after client contact the antibacterial soaps can remove 65 to 85% bacteria from human skin. Some of the microbes cause disease on the skin while the others become pathogenic only when they migrate to mucous membrane and other vital organs, *S. aureus* has been known to be one of the many dangerous and versatile pathogens with high death rates in human as a consequence of community acquired and hospital-acquired infections.

The process saponification, which yields soap, involves combining acids in the form of vegetable or animal fats with an alkali (lye, a solution of sodium hydroxide and water) to create a mixture of soap and glycerine. To put it simply, it's a slick material that releases bubbles. Making soap is a traditional skill from ancient times. It is possible that soap was found by prehistoric people when fat and ash came together and saponified while cooking over open pits. In the Pompeii remains, a soap factory was discovered, and Greek physician Mr. Galen wrote about soap in the second century. It's amazing to think that soap needed to have its intended usage labelled as recently as the previous century. Moreover, soap lost favour at this time because some modest Victorians thought "soap baths" were shameful and immoral. In actuality, several of the well-known brand-name soaps you purchase today aren't even soap. The items are referred to be "beauty bars" or "deodorant bars" on the packaging rather than "soap." These detergents are artificial. Additionally, these "beauty bars" include chemical elements referred to be "builders" that boost the soap's effectiveness.

There are many natural ingredients which are easily available against *S. aureus* such as *Achyranthes aspera*, *Acalypha indica*, *Azadirachta indica*, *Senna auriculata*, *Curcuma aromatica*.

1.1 NATURAL INGREDIENTS

1.1.1 *Azadirachta Indica*

Neem (*Azadirachta indica*), also known as Nim or Margosa, is a fast-growing tree of the mahogany family (Meliaceae) that is prized for its timber, medical properties, and use as a source of natural insecticides. 1. Neem is most likely indigenous to South Asia's drier regions, including the Indian subcontinent. It has been brought to several nations in South and Central America, the Caribbean, and areas of Africa. The plant has a long history of usage in Ayurvedic and traditional medicine, cosmetics, and organic farming (Wylie MR, Merrell DS 2022).



Fig:1.1.1: *Azadirachta Indica*

1.1.1.1 Plant Specifications

Neem trees range in height from 15 to 30 meters (49 to 98 feet), and they have visually pleasing rounded crowns with thick furrowed bark. Normally evergreen, the intricate leaves have serrated leaflets, but in periods of excessive dryness, they may fall off. The little, fragrant white blossoms, which are male or staminate (bisexual), are borne in clusters in the leaf axils. The fruit's flesh is a smooth, yellow-green drupe with a nice flavour. Neem can be multiplied by cuttings or root suckers; however, seeds are frequently the recommended approach. Because the plant is hardy and persistent, it can survive in rocky, poor soil. Neem is resilient to a wide variety of weather conditions; however, it is not able to tolerate freezing temperatures or flooding.

Azadirachta Indica, commonly known as neem or Indian lilac, belongs to the Maliaceae family and is classified under the order Sapindales within the Kingdom Plantae.

Although it was originally from the northeast of the Indian subcontinent and Indochina, it has now become naturalized and is now cultivated in tropical and subtropical regions all over the world. Neem oil is produced from the plant's fruits and seeds. Neem is a Hindustani noun that is derived from the Sanskrit word nimba.

1.1.1.2 Chemical constituents:

Neem, *Azadirachta indica*, has a therapeutic role in the management of health since it is a rich source of many different kinds of substances. Azadirachtin is the most significant active component, followed by sodium nimbinate, nimbolin, nimbin, nimbidin, nimbidol, gedunin, salannin, and quercetin. The compounds nimbin, nimbanene, 6- desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, 7-desacetyl-7-benzoylazadiradione, 17-Hydroxy azadiradione, and nimbiol are all found in leaves. Neem fresh leaves were used to extract the polyphenolic flavonoids quercetin and β -sitosterol, which are known to have antibacterial and antifungal activities. Neem seeds also contain important compounds including gedunin and azadirachtin.

1.1.1.3 Medicinal Uses

Antifungal activity A fungal affliction Since time immemorial, people have thought that Neem is effective against certain fungi that infect the human body. Neem preparations have been discovered to be effective against a number of significant fungi, including athlete's foot fungus, which affects the skin, hair, and nails, ringworm, which attacks the skin and nails of the feet, fungi that grow in the lungs, bronchi, and mucous membranes, and fungi that are normally found in the mucous membranes but can become out of control and cause lesions in the mouth (thrush), vagina, etc. Neem leaf and oil seed extracts are effective against a number of fungus, including Candida, Trichophyton, Epidermophyton, Microspore, Trichosporon, and others. Effect on central nervous system, Anti-diabetic effect, Anti-oxidant effect, Anticancer effect, Antiviral activity, Antibacterial activity, Antimalarial activity, Antiulcer effect, Hypoglycemic activity, Immunostimulant activity.

1.1.2 *Achyranthes aspera*

Achyranthes aspera (common names: chaff-flower, prickly chaff flower, devil's horsewhip, is a species of plant in the family Amaranthaceae. It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. It is an invasive species in some areas, including many Pacific Islands environments (Barua CC *et al.*, 2012).



Fig: 1.1.2: *Achyranthes aspera*

1.1.2.1 Plant specifications

The perineal wild grass *Achyranthes aspera* grows straight. Its stems are herbaceous above but woody below; they are erect, branched, cylindrical, solid, angular, hairy, and longitudinally striated. They are green at internodes and nodes but violet or pink at nodes. Their leaves are simple, exstipulate, opposite decussate, petiolate, whole, ovate or obovate, acute or acuminate, hairy throughout, and unicostate reticulate. An inflorescence is a spike on a long peduncle bearing reflexed blossoms. Harmonious, sessile, actinomorphic, pentamerous, hypogynous, small, spinescent, green, with two bracteoles that are shorter than the perianth and are dry, membrane, and persistent flowers. Ovate, awned bracts, persistent. Gynoecium: single and filiform style, stigma capitate, basal placentation, ovule one, bicarpellary, syncarpous, superior, and unilocular. oblong utricle of fruits. Endospermic seeds have an oblong, black, curving embryo that is 2 mm long. Perianth wrote the piece. The species *Achyranthes aspera* belongs to the genus *Achyranthes*, which is classified under the family Amaranthaceae, within the order Caryophyllales, in the clade of Angiosperms within the kingdom Plantae.

1.1.2.2 Chemical constituents

The *Achyranthes aspera* is stated to contain the following major classes of compounds they are Fatty acids, D-glucuronic, Betaine, Oleanolic acid, triacontanol, Spathulenol, alkaloids, Achyranthine, different amino acids, Ecdysterone, Oleonic acid, Bisdesmosidic, triterpenoid-based saponins, Spina sterol, dihydroxy ketones, n-hexacos-14-enoic are present.

1.1.2.3 Nutritional constituents

Achyranthes aspera plant and seeds are rich in carbohydrates, proteins, and bioactive constituents such as flavonoids, tannins, and saponins that help maintain an individual's overall health.

1.1.2.4 Medicinal uses

The pulp of fresh *Achyranthes aspera* leaves, and flowering spikes is an effective home remedy for scorpion bites when applied externally, which is believed to paralyze. For snake/reptile bites, the seeds are beneficial for ophthalmic diseases and other corneal infections.

Flowering, elongated spikes mix with little sugar and used in mad dog bites to treat hydrophobia. *Achyranthes aspera* ash loaded with potash has been used for washing clothes. Flowers and fruits of *Achyranthes aspera* are useful for the treatment of menorrhagia. All parts (seeds, roots, leaves) of *Achyranthes aspera* have been used both traditionally and medicinally; it has a strong unpleasant smell used externally or internally. The *Achyranthes aspera* having expectorant, stomach tonic, laxative, anthelmintic, diuretic, lithotriptic, sudorific, demulcent, anti-inflammatory, anticataract, antifungal, antibacterial, hypoglycemic, antihyperlipidemic, antiperiodic, antiasthmatic, hepatoprotective, anti-allergic properties it aids to treat various health issues. *Achyranthes aspera* plant supplements help remove excess body fat by preventing accumulation in the abdomen; gluteal regions control body weight. *Achyranthes aspera* juice has ropan (astringent), anti-inflammatory properties; therefore, it is used in ulcers/wounds for faster healing. *Achyranthes aspera* root paste and juice help to lower the pain, reduce itching, skin rashes in insect bites, and urticaria when applied topically and helpful in earache. Daily consumption of *Achyranthes*

aspera supplement powder with honey improves digestion. *Achyranthens aspera* has excellent digestive and appetizing properties, with the help of keeping digestion to keep the body healthy. In Ayurveda, a unique medicine called *Achyranthens aspera* (Apamarga ash) is very effective externally for fistula and fissure. *Achyranthens aspera* oil is predominantly used to expel excessive cough in the body & gives relief from cough. *Achyranthens aspera* has purgative quality, which helps to softens stool and lowers the risk of constipation, piles, fistula/fissures. When *Achyranthens aspera* taken orally breaks down calcium oxalate calculi by its Tikshna property, it expels out by increasing urine. *Achyranthens aspera* is rich in potassium, so it keeps the heart-healthy.

1.1.3 *Acalypha Indica*

Acalypha indica (English: Indian acalypha, Indian mercury, Indian copperleaf, Indian nettle, three-seeded mercury) is an herbaceous annual that has catkin-like inflorescences with cup-shaped involucre surrounding the minute flowers. It is mainly known for its root being attractive to domestic cats, and for its various medicinal uses. It occurs throughout the Tropics (Zahidin NS *et al.*, 2017).



Fig: 1.1.3: *Acalypha indica*

1.1.3.1 Plant specifications

It grows in disturbed areas including roadside ditches, wall cracks, and waste areas. It thrives along riverbanks, woodland borders, and rocky hillsides as well. It favors areas that are damp and dark. It grows from sea level to an altitude of 1350 meters. An upright annual herb that is easily identified by the small flowers in the inflorescence that resemble catkins, which are surrounded by a cup-shaped involucre. When conditions are right, it can reach a height of 1.2 m (3.9 ft),

although it normally grows shorter. Broad ovate leaves measuring 1.2 cm–6.5 cm × 1 cm–4 cm (0.47 in–2.56 in × 0.39 in–1.57 in) are present. The base of the leaf is rounded to attenuate quickly. The leaf margin has an acute or obtuse apex, is crenate-serrate, and is basally 5-nerved. The petiole measures 0.59–2.17 inches, or 1.5–5.5 cm. The flower spikes are monoecious, axillary, and 4-6 cm (0.98-2.36 in) long. The rachis ends in a triradiate hood. The tiny, white-green male flowers are ebracteate, minute, and grouped with varicelliform anthers. They are situated on the upper portion of the flower spikes. The pollen grains have a diameter of 10–12 microns and are essentially spherical.

The species *Acalypha indica* belongs to the genus *Acalypha*, which is classified under the subtribe Acalyphinae, within the family Euphorbiaceae, in the order Malpighiales, within the clade of Rosids among the Eudicots, which are Angiosperms, belonging to the larger clade of Tracheophytes within the kingdom Plantae.

1.1.3.2 Chemical constituents

The arial parts contain a cyanogenic glycoside called acalyphin (a 3-cyanopyridone derivative) as well as flavonoids, such as kaempferol glycosides mauritianin, clitorin, nicotiflorin, and biorobin. Tannins, β -sitosterol, acalyphamide, aurantiamide, succinimide, and flindersin (a pyranoquinolinone alkaloid) have also been isolated. The chemicals that attract cats are the iridoid compounds isodihydronepetalactone and iso-iridomyrmecin.

1.1.3.3 Medicinal uses

Anti inflammation property, Analgesic property, Anthelmintic property, Anti-bacterial property, Anti-venom property, Wound healing property, Anti-ulcer property, Anti-diabetic property, Larvicidal and ovicidal property, Anti-oxidant property, *Acalypha indica* is used to get rid of unwanted hair growth, for treating skin problems like pimples, psoriasis and eczema, to get rid of cough and cold, to treat intestinal worms and also for treating piles.

1.1.4 *Senna auriculata*

A leguminous tree belonging to the Caesalpinioideae subfamily is called *Senna auriculata*. Local names for it include mature tea tree, avaram or ranawara, and the English translation is avaram senna. It is the official flower of the Telangana state in India. It is found in the arid regions of Sri Lanka and India. In Sri Lanka, it is typical in the dry zone and along the coast. (Nille GC *et al.*, 2021)



Fig: 1.1.4: *Senna auriculata*

1.1.4.1 Plant specifications

The Avaram senna is a densely branching shrub with closely spaced pubescent branchlets and smooth, cinnamon coloured bark. Leaflets 16–24 are very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate, globous or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at base on side of next petiole into a filiform point and persistent. The leaves are alternate, stipulate, paripinnate compound, very numerous, closely spaced, rachis 8.8–12.5 cm long, narrowly furrowed, slender, pubescent, and arranged. Its huge, irregular, bisexual, bright yellow blooms measure almost 5 cm wide. Its 2.5 cm long, globous pedicels complement the flowers. The ovary is superior, unilocular, with marginal ovules; the racemes are few-flowered, short, erect, and packed in the axils of the higher leaves to produce a huge terminal inflorescence stamens barren. The fruit is a short legume that is flat, thin, papery, undulate crimped, pilose, pale brown, and measures 7.5–11 cm long by 1.5 cm broad. It is also oblong and obtuse. Each fruit has 12–20 seeds, each of which is carried in a distinct cavity. The species *Senna auriculata* belongs to the *Senna*, which is classified under the subfamily Caesalpinioideae, within the family Fabaceae, in the order Fabales, within the

clade Rosids among the Eudicots, which are Angiosperms, belonging to the larger clade of Tracheophytes within the kingdom plantae.

1.1.4.2 Chemical constituents

Avarampoo is a natural source of antioxidants. The extracts of its flower contain terpenoids, tannins, saponins, cardiac glycosides and flavonoids. Helps Treat Diabetes: The flower also has anti-diabetic activity. Glycine, dodecanoic acid, linoleic acid, oleic acid, grape seed oil, stearic acid, palmitic acid, dl- α -tocopherol, stigmasta, dimethoxy acetophenone, fatty acid, and fatty acid ester. *C. auriculata* flower extract contains flavonoid and phenols in a significant amount.

1.1.4.3 Medicinal uses

The plant has been reported to treat hyperglycaemia and associated hyperlipidaemia. This plant is said to contain a cardiac glucoside (sennapicrin) and sap, leaves and bark yield anthraquinones, while the latter contains tannins.

The root is used in decoctions against fevers, diabetes, diseases of urinary system and constipation. The leaves have laxative properties. The dried flowers and flower buds are used as a substitute for tea in case of diabetes patients. It is also believed to improve the complexion. The powdered seed is also applied to the eye, in case of chronic purulent conjunctivitis. In Africa the bark and seeds are said to give relief in rheumatism, eye diseases, gonorrhoea, diabetes and gout. The plant has been shown to have antibacterial activity in the laboratory.

1.1.5 *Curcuma aromatica*

Curcuma aromatica, sometimes known as wild turmeric, is a plant in the Zingiberaceae family's genus *Curcuma*. Wild turmeric, which is botanically related to *Curcuma australasica*, has been utilized extensively throughout South Asia and the surrounding areas as a cosmetic plant. It goes by the name Kasthuri Manjal in Tamil and Malayalam (Albaqami JJ *et al.*, 2022).



Fig: 1.1.5: *Curcuma aromatica*

1.1.5.1 Plant specifications

One of the 80 plants in the Zingiberaceae family is wild turmeric. The rhizomes stay dormant throughout the winter, and the perennial foliage eventually dies out in late autumn. Early spring sees the appearance of the flower at the rhizome base. Throughout the summer monsoon season and the weeks that follow, the plant develops quickly and furiously. The stalk reaches a height of approximately 20-30 centimetres (7.9-11.8 in) and is topped with larger, pink-tip bracts that are coloured. Frequently, leaves emerge even after the blooms. The plants can grow up to be around 40 cm (16 in) tall when fully grown. *Curcuma aromatica*, also known as wild turmeric or aromatic turmeric, is a flowering plant belonging to the Zingiberaceae family within the order Zingiberales. It is classified as a monocot, a group of angiosperms characterized by having one seed leaf. Within the kingdom Plantae, it falls under the clade of tracheophytes, which are vascular plants with specialized tissues for conducting water and nutrients. This species is part of the Commelinids clade, which includes grasses and related plants. Its genus, *Curcuma*, comprises perennial rhizomatous herbs known for their culinary and medicinal uses.

1.1.5.2 Chemical constituents

The rhizomes of wild turmeric contain curcuminoids (curcumin, desmethoxycurcumin, and bisdemethoxycurcumin) and essential oils including xanthorrhizol, ar-curcumene, di-epi- α -cedrene, turmerone, ar-turmerone, zingiberene, β -sesquiphellandrene, germacrone, 8,9-dehydro-9-formyl-cycloisolongifolene, furanodienone.

1.1.5.3 Medicinal uses

Wild turmeric has rhizomes with a peculiar fragrance and cream colour. The rhizomes are often used as a culinary ingredient, and in traditional medicine, for skin disorders and as an antibacterial agent. It is also commonly used in ethnic cosmetic products. As a culinary ingredient it is used in limited quantities as a natural food colour. Leaves are broad and very decorative, elliptic with a leaf stem running as long to the tip of the blade. A fresh stalk with flowers and leaves, cut to proper size and shape, can be used as a floral indoor decoration in vase for up to 10 days.

CHAPTER 2

LITERATURE SURVEY

The extensive literature survey was performed using database platforms to study regarding the formulation and evaluation of antiseptic properties displayed by herbal soap. The study showed that formulation of herbal soap was done by utilizing *Matri Caria chamomilla* flowers along with their evaluation of antimicrobial property effective against *S. aureus* and *P. aeruginosa* skin pathogen and performed by using the disc diffusion method. Efficacy of the synthesized soap was checked and compared to the commercially available soap. Results showed that the herbal soap was more efficient than commercial soaps in reducing the number of microorganisms (Heyam *et al.*, 2019). The another research revealed that formulation and evaluation of nontoxic herbal hand-wash by using barks of the *Madhuca indica*. Evaluation of antimicrobial activity was done by disc diffusion methods and results revealed that synthesized formulation was more efficient than industrial antiseptic soaps (Sharma *et al.*, 2003).

Joshi et al., study was about evaluation of herbal soap formulation. Evaluation of antimicrobial efficacy of the herbal soap was done by glove juice method and it revealed that synthesized herbal soap was more proficient than commercially available soap in reducing the number of organisms. The study showed the formulation and evaluation of Herbal soap by using ethanolic extract of *Glycyrrhiza glabra* root. Disc diffusion method was used to evaluate the antimicrobial activity of synthesized herbal soap and its efficacy was evaluated and compared with the standard commercial soaps available. Results showed that the synthesized herbal soap using extract of *Glycyrrhiza glabra* root was more efficient than commercially available soaps in reducing the organism with no side effects (Patel *et al.*, 2017).

And another study revealed Formulation and evaluation of poly herbal hand-made soap which contains essential oils. Pour plate technique was used to evaluate the antimicrobial activity of synthesized poly herbal gel-based soap. Results showed that the synthesized herbal soaps were more proficient than commercially available soap in reducing the organism with no side effects (Mounika and Jyothi 2022).

The research showed the formulation and evaluation of poly herbal antibacterial gel-based soap using the *Garcinia indica* and *Curcuma longa* extract. Antimicrobial activity of formulated poly herbal antibacterial gel-based soap was evaluated against skin pathogen and its efficacy was tested using disk diffusion method. Results concluded that utilization of herbs in the formulation of soap give a better effect with less or no side effects (Power *et al.*,2018).

The formulation and evaluation of herbal soap by using natural ingredients by simple matched, the herbal soap was formulated by using leaf of neem, Aloe Vera, and Tulsi ayurvedic cosmetics is very helpful and does not give it side effects. Ayurvedic cosmetics are also known as herbal cosmetics. All herbal ingredients are easily available market of surrounding areas. Munde Govind Anant *et al.*, (2021) and Evaluation of butterfly pea flowers for antioxidant activity and its potential as antioxidant soap, *Clitoria ternatea*, often known as butterfly pea, is a flowering vine plant in the Fabaceae family with oval and acute leaves. Due to free radicals, many severe diseases were occurred such as cancer, diabetes, and cardiovascular and neurological illnesses. Aside from that, if the cellular antioxidant defence mechanism is depleted, free radicals can easily spread. Increasing the antioxidant defence layer in the human body system will eventually aid in lowering free radicals from harming cellular components (Nur Amira Abd Rashid *et al.*, 2022).

The studies on antimicrobial activity of antiseptic soaps and herbal soaps against selected human pathogens, In the present study antimicrobial activity of various antiseptic and herbal market soaps were determined against bacterial isolates present on the skin surface like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* using agar disc diffusion method. Various microbes are deposited on the surface of skin from the dust present in external environment which causes infection. Antimicrobial activities of various soaps on such micro flora pathogens were studied (Varsha M Chaudhari *et al.*, 2016).

The effects of cold saponification on the unsaponified fatty acid composition and sensory perception of commercial natural herbal soaps, Saponification is the process in which triglycerides are combined with a strong base to form fatty acid metal salts during the soap-making process. The distribution of unsaturated and saturated fatty acid determines the hardness, aroma, cleansing, lather, and

moisturizing abilities of soaps. Plant extracts, such as rosemary, vegetable, and essential oils are frequently added to soaps to enhance quality and sensory appeal. Three natural soaps were formulated using cold saponification to produce a base or control bar (BB), hibiscus rosehip bar (H), and a forest grove bar (FG). Rosemary extract (R) or essential oil (A) blends were added as additives to each formulation prior to curing to evaluate the effects of natural plant additives on the lipid composition and sensory characteristics of these natural herbal soaps (Natalia Prieto Vidal *et al.*, 2018).

The formulation of herbal soap against acne causing bacteria, the use of herbs on skin disorders has been done for thousands of years. Herbal remedies, including those for many kinds of disorder especially skin disorders, are currently gaining popularity among patients. In Asia, especially in south East Asian countries, herbal treatments that have been used from ages are now being studied scientifically. In India, records of Ayurvedic medicine, a method of medicine with natural roots date back to about 3000 BC (Anjum *et al.*, 2021).

Antibacterial Chemical Constituent and Antiseptic Herbal Soap from *Salvinia auriculata*, in recent decades, research on plants with antimicrobial properties has intensified, due mainly to the fact that these plants are considered sources of pharmacologically active compounds that can become new antibiotics after their pure constituents or active extracts have been evaluated. Plants that live in nutrient-rich environments, as well as plants living in environments containing exceptionally high bacterial cell density (i.e., aquatic plants), will be overwhelmed by microbial biofilms if they lack any means of biofilm control (Samia Lima 2013).

The prevalence of drug use in institutionalized elderly people: a descriptive study, the process of demographic transition is a reality that has been changing the profile of the population and morbimortality, resulting, among other things, In the aging of the population. The information made available by the last national sense by the Brazilian Institute of Geography and Statistics (BIGS) highlights the extension of the top of the age pyramid, which rose from 4.8% in 1991 and 5.9% in 2000 to 7.4% of elderly population in general in 2010. Although the National Policy for the Elderly and the Elderly Statute preconize the maintenance of the elderly among their families, one of the results of this demographic transition is the increased demand by the elderly for long-stay institutions (ILPIs), growing

alternative related to factors such as: terminal stages of disease, high dependency of the elderly, higher emergence of chronic diseases (Marielli *et al.*, 2012).

Cypirus rotundos I formulation and evaluation antiseptic soap, soap is a product that is one of the people's needs, used as a body cleansing agent, which is available in many forms and perfume that can be selected according to your needs. Natural soap products are rarely used in the market. Today's soap products use a lot of artificial ingredients as their active ingredients, which have side effects on human skin because they have the potential to irritate users with sensitive skin. This study aims to describe the antiseptic soap formula and evaluate the antiseptic soap from c. rotundos (Sri Dwiyanti 2021).

The Influence of the Addition of the Essential Oil of Cinnamon (Cinnamomum burmanii) in Soap Against Skin Care, Cinnamon contains the compound eugenol 3.11% and cinnamaldehyde (90.24%) and coumarin (53.46%), which can reduce acne blemishes on an oily face and have a thinning effect on stain and acne busting speed. In addition, cinnamon also contains vitamin C that plays and protects the skin from UV rays from bad influences that cause premature aging and prevent the formation of melanin. Cinnamon can be processed into oil through distillation. The Cinnamon essential oil can be mixed in cosmetic products such as herbal soap and utilized for skin care. For this research, the herbal soap was developed with a mixture of cinnamon oil. The process began from the preparation of materials by processing Cinnamon Bark essential oil into cinnamon and mixing with essential oils in the formula herbal soap to become a soap product (Dwi Atmanto *et al.*, 2019).

The Preparation of soap using a steam extraction process from leaves of *Azadirachta indica*, *Ocimum basilicum*, *Hibiscus rosasinensis* Flowers, *Acalypha indica*, and Aloe barbadense Leaflets, Soaps are used widely by humans in many aspects. Exclusively, the emergence of the SARS-Cov-2 virus made people wash their hands frequently to disinfect the virus to prevent virus infection. Soaps made of herbals possess constituents that goodness the skin as well to rejuvenate the mind and body factors. Dried leaves of Basil, Neem and *Acalypha Indica*, Aloe vera, and Hibiscus flower are used here to prepare homemade soap. The steam distillation process is an imperative process by which the oils are used from raw materials like dried leaves for the preparation of soaps (V S Kavinkumar *et al.*, 2007).

2.1 NATURAL INGREDIENTS USED IN HERBAL SOAPS SO FAR

Vitex negundo, *Terminalia catappa*, *Curcuma longa*, *Garcinia indica*, Atriplex roots, Sapindus fruits, Mojave yucca root, Soapwort root, Buffaloberry fruits, Neem, Aloe vera, Honey, Almonds, Jasmine, Lemon, Strawberry and Sandalwood (Sucharita G, *et al.*, 2021).

2.2 HISTORY OF SOAPS

The first recorded evidence of the manufacture of soap-like materials dates back to around 2800 BC in Ancient Babylon. Babylonians discovered the basic method of making soap (fats boiled with ashes and water). Soap was used mostly in the textile industry (Sudharani M V, *et al.*, 2014).

The Ebers papyrus (Egypt, 1550 BC) describe how animal and vegetable oils were mixed with alkaline salts to produce a soap-like substance and mention that soap was used for threatening sores, skin diseases as well as washing (Kumar Sudheer K *et al.*, 2020).

The ancient Greeks were known to wash without soap. They preferred to wash with water, blocks of clay, pumice, sands and ashes and then to anoint themselves with oil. In the second century AD, Galen, the famous Greek physician, recommended washing with soap as a preventive measure for skin diseases. Ancient Germans and the Gaul's mixed ashes with animal fat to produce soap, and they used it to decorate their hair (Chavan *et al.*, 2019).

Arabic chemists were the first one to produce soaps made from vegetable oils (such as olive oil), aromatic oils (such as thyme oil) and lye. From the beginning of the 7th century, soap was produced in Nablus, Kufa and Basra. They made perfumed and coloured soap, some of the soaps were liquid and others were solid. They also made special soap for shaving.

By 1200 AD, Marseilles, France and Savona, Italy became soap making centres. In the 8th century, soap making was well-known in Italy and Spain where soap was made with goat fat and Beech tree ashes. During the same period, the French started using olive oil to produce soap. Eventually, fragrances were introduced and soaps for bathing, shaving, shampooing and laundry began to be made. From the 16th century finer soaps were produced in Europe using vegetable oils (such as olive oil) as opposed to animal fats. Many of these soaps are still manufacture, both industrially and by small scale soap makers.

Due to a better understanding of the role of hygiene and the promotion of popular awareness of the relationship between cleanliness and health, industrially manufactured bar soaps became available in the late eighteenth century. The big leap in commercial soap making was two discoveries by French chemists Nicholas Leblanc and Michael Chevreul around the turn of the 19th century. In 1791, Leblanc patented a method of making sodium carbonate or soda ash from commonly available salt. In 1811, Chevreul discovered the relationship and chemical nature of fatty acids, glycerine, and fats. Andrew Pears began manufacturing a high-quality, transparent soap in 1789 in London. William Gossage manufactured low-price good-quality soap from the 1850s. Robert Spear Hudson started producing a soap powder in 1837.

The development of synthetic detergents in Germany in 1916 made another breakthrough in soap making chemistry. Another well-known soaping centre was The Castille region of Spain, responsible for making the first, hard, white bars of olive oil soap. Castille soap is still known today like soaps made with all, or mostly all, olive oil. Commercial soap, as we know it today, came into existence during WWI. After the Great War and until 1930's, a method called batch kettle boiling was used for soap production. Shortly thereafter, continuous process that decreased soap making production time to less than a day was introduced and refined by Procter & Gamble. Continuous process is still used by large commercial soap manufacturers. (Talreja *et al.*, 2021).

2.3 THE SKIN AND THE NORMAL SKIN MICROBIOTA

Skin microbiota, another name for skin flora, is the word used to describe the microorganisms that live on skin, usually human skin. There are around 1,000 different species of bacteria on human skin belonging to nineteen different phyla (Kong, 2015) the majority of them are found in the higher regions of hair follicles and the superficial layers of the epidermis. The majority of skin flora is non-pathogenic it is either mutualistic, which means it benefits the host, or commensal, which means it does not damage it. Bacteria can be beneficial because they can compete with temporary harmful organisms for nutrition, secrete substances that inhibit them, or boost the skin's defences against them (Cogen, 2016). However, local bacteria can enter the bloodstream and cause skin infections, leading to serious illnesses, especially in patients with immunosuppression (Grice, 2015).

Batrachochytrium Dendrobatids, a chytrid and non-hyphal zoospores fungus, is a prominent component of the skin flora in non-human animals. It is believed to be the source of chytridiomycosis, an infectious disease that is responsible for the fall in amphibian populations (Jamie, 2018). There are folds, invaginations, and specific niches in the 1.8 m of human skin. Numerous types of microbes live in this habitat, which gives them food and shelter (Serge, 2019). The major function of the skin, which is a component of the human innate immune system, is to act as a physical barrier, shielding our bodies from potential threats from harmful substances or foreign organisms. It is the initial line of defence against infectious pathogens that invade the body (Akanbi, 2018). The majority of pathogenic pathogens cannot pass through the skin's epithelial surfaces, and skin epithelium desquamation also aids in the human body by eliminating germs and other harmful organisms that have stuck to epithelial surfaces. On the other hand, infection can lead to disease when the skin is broken (Akanbi, 2018). As the skin serves as a barrier between the inside and outside of the body, a wide variety of microorganisms, including commensals, parasites, and symbiotes, populate it. These consist of mites, bacteria, fungus, and viruses (Haller, 2017). Many of these microorganisms are benign, and the symbiotes may occasionally perform essential tasks for which the human genome has not evolved (Yusuf, 2015). They may also prevent the invasion of more pathogenic or harmful organisms by competing with them for nutrients and adhesion proteins on the skin, producing substances that are toxic to them, or both. of skin-resident T cells, preparing them to react to their similarly marked pathogenic cousins (Grice, 2019). These microbes fall into one of two groups: pollutants (also known as "transient flora") (Okon, 2015). According to Okon claims that Gram-positive cocci and diphtheroid make up the majority of the typical flora (also known as "resident flora") on the skin, which may act as a selective barrier to prevent the growth of potentially harmful organisms. This typical flora may also include minor amounts of yeast or Gram-negative organisms in some individuals, but the regular ecological balance may have an impact on how quickly these species proliferate (Okon, 2017).

2.4 CLINICAL MANIFESTATIONS AND PATHOGENESIS OF *STAPHYLOCOCCUS AUREUS*

The versatility of *S. aureus* is seen in a variety of clinical conditions which include: septicaemia, pneumonia, wound sepsis, septic abortion, osteomyelitis, septic arthritis, post-surgical infections and toxic shock syndrome (Nkwelang, 2015).

These results in high rates of morbidity and mortality in humans worldwide (Murray, 2014). Approximately 30% of healthy individuals can asymptotically carry *S. aureus* on mucous membranes for weeks or months but is only transiently carried on the intact skin. Five stages are seen in the pathogenesis of *S. aureus*. These include:

2.4.1 Colonization

According to the Gale Encyclopedia of medicine is the presence of bacteria on a body surface (like on the skin, mouth and intestines without causing disease in the person. Infection is the invasion of a host organism's bodily tissues by disease-causing organisms.

2.4.2 Local infection

A local infection is an infection that has not spread but remains dormant or contained near the entry site.

2.4.3 Systemic dissemination

This is an infection that is localized and disseminates from one area of the body to other organ systems. While there are systemic infections that can affect the entire body at once, doctors will reserve the term for those infections that are normally constrained to a specific site.

2.4.4 Metastatic infection

This infection is known to be a deep-seated infection, it includes endocarditis and muscle blister, detected within 3 months after the initial positive blood culture result was obtained.

2.4.5 Toxicosis

This is a pathological condition that can be caused by the action of either a poison or a toxin. Once in blood, the organism spreads widely to peripheral sites in distant organs and septic shock can occur (Shittu, 2018). Without specific therapy, the mortality rate resulting from spread of infection is high. Specific infections result from haematogenous dissemination, these include: endocarditis, osteomyelitis, renal carbuncle, septic arthritis, or epidural abscess (Shittu, 2015). Even when the organism itself does not invade the bloodstream, specific syndromes such as toxic shock syndrome, scalded skin syndrome and foodborne gastroenteritis result from the local or systemic effects of specific toxins (Archer, 2019).

2.5 EARLY SOAP MAKING

Ancient Mesopotamians were first to produce a kind of soap by cooking fatty acids like the fat rendered from a slaughtered cow, sheep or goat together with water and an alkaline like lye, a caustic substance derived from wood ashes.

2.6 MODERN SOAP MAKING

There are many soaps available now like organic soaps, inorganic soaps, etc., molten fat and water are introduced continuously into opposite ends of the column, fatty acids and glycerine are simultaneously withdrawn. Next, the fatty acids are distilled under vacuum to effect purification. They are then neutralized with an alkali solution such as sodium hydroxide (caustic soda) to yield neat soap.

2.7 PROPERTIES OF SOAP

There are some specific properties that make soap a high-quality soap product. Cleansing is the ability of soap to remove dirt and oil from the skin. It is the most critical property of soap, as it is why most people use soap in the first place. Good cleansing requires two elements

2.7.1 The ability to remove dirt and oil

The ability to clean without stripping the skin of its natural oils. The most effective soaps can also remove water-soluble and oil-soluble.

2.7.2 Hardness

Hardness is an essential property of soap because it determines its duration. If soap is too soft, it does not last long, so users need frequent replacement. On the other hand, a too-hard soap is difficult to use and may cause skin irritation.

The perfect hardness for soap is somewhere in the middle. The number of fatty acids in the soap determines the soap's hardness. The fattier acids, the more complex the soap.

2.7.3 Lather

When soap mixes with water, bubbles and foam form the soap's lather. The lather is essential because it helps remove dirt and oil from the skin. A soap that creates much lather is usually more effective at cleansing.

2.7.4 Fragrance

A good-smelling soap is more appealing to use. When choosing a soap, it is essential to consider the fragrance. Some people prefer a strong odour, while others prefer a light scent. The type of fragrance used in soap is also necessary. Some scents, such as essential oils, can benefit the skin, while synthetic fragrances may cause skin irritation.

2.7.5 Conditioner

Conditioners are ingredients that make the soap gentler on the skin. Standard conditioners include glycerine, honey, and oatmeal. These ingredients help to moisturize the skin and make the soap gentler.

2.8 TYPES OF SOAP

There are many types of soaps, all created using different methods. Soap can be made from scratch by a cold or hot process, or might chose a ready-made soap base.

2.8.1 Cold process soap

The method most used by those preparing soap from scratch. It is called "cold process" because no additional heat is added during the soapmaking

process. However, the process itself does generate heat. Soaps produced via the cold process method are opaque and usually have a creamy feel to the bar. Without any additives that change the colour, the soap ranges from white to creamy-tan, depending on the oils used in making the soap. Most cold process soaps are made with a combination of oils, in a recipe developed by the soap maker to create a good lather and hard bar, as well as to provide benefits with additional ingredients.

2.8.2 Hot process soap

Additional heat is applied to the soap mixture during the hot process method. The chemical reaction is the same but occurs much faster than cold process and the heat typically generates a more “rustic appearance”. Hardness depends oils, amount of water used, and time allowed for water evaporation. As with cold process, hot process soap is opaque and ranges from white to creamy-tan depending on the oils used, although clear or transparent soaps can be produced. The type and quality of the lather and other benefits of the soap are determined by the oils and other ingredients selected chosen for the recipe.

2.8.3 Liquid soap

Liquid hand soap is the most common type of soap you’ll see in homes and businesses for handwashing. You can find cheap liquid soap or even luxury quality. It doesn’t just stop at liquid soap, though. Different types of liquid hand soap have various purposes.

2.8.4 Moisturizing soap

Moisturizing soap has a moisturizing agent that helps moisturize your hands as you wash them. Frequent handwashing every day can dry out your hands, but it’s necessary. Some examples of moisturizing agents in these soaps are aloe, cocoa butter, and shea butter. So, if you notice your skin especially dry after handwashing, a moisturizing soap may help.

2.8.5 Antibacterial soap

Antibacterial soap has become increasingly popular because it kills all the bacteria on your hands if adequately used. However, research shows that

antibacterial soap is no better at removing germs than other liquid hand soap. However, healthcare facilities often use antibacterial or antimicrobial soap, which is effective and necessary in those settings.

2.8.6 Natural soap

Natural hand soap uses natural ingredients like essential oils and leaves out harsh chemicals like parabens, sulphate, phthalates, artificial fragrances, and synthetic dyes. Natural hand soaps are just as effective as regular hand soap in getting rid of germs through proper handwashing. It usually costs more than conventional hand soap, but it's a good middle ground between regular hand soap and completely chemical-free hand soap.

2.8.7 Chemical free soap

Most hand soaps on the market have a chemical detergent in their ingredients. Even a hand soap that's considered "natural" usually has a chemical detergent along with its natural ingredients. A chemical-free soap uses plant-based ingredients and is made with oil and lye. These hand soaps can get pricey, but it's a necessary investment for some. On the other hand, plant-based and chemical-free soaps might be necessary if you have extra sensitive skin, and sometimes it's a lifestyle choice. Regardless, chemical-free soap can still be effective at removing germs as other hand soaps.

2.8.8 Foam soap

Foaming hand soap is just liquid soap pushed through a unique dispenser to give it the foam texture. One of the benefits of foam hand soap is that it provides more of a lather, making it easier to wash your hands. In addition to regular foam soap, you'll find foam hand soaps that are all-natural, antibacterial, and moisturizing. (Achaw, Osei-Wusu *et al.*, 2021).

2.9 BENEFITS OF SOAP

2.9.1 Nourishing

Herbal soaps contain the natural goodness of nature, and this makes them nourishing for your skin. It offers comfort, healing and stress relief as well.

2.9.2 Therapeutic

Because of properties such as relief from tension and the healing power of herbal soaps, the benefits are there in plenty for those seeking to rejuvenate the mind, body, and spirit.

2.9.3 Aromatic

Herbal soaps have an aroma which makes them extra special. It has a selection of exclusive elements and is devoid of artificial colour and fragrance. Moreover, chemical soaps have animal fat and lack the essential oils from plant extract which give a natural and pleasing aroma.

2.9.4 Relief from Skin Diseases

Handmade herbal soaps have therapeutic benefits in addition. Those with sensitive skin or conditions such as psoriasis or eczema can take aid from natural handmade soaps. Glycerine soap also protects the skin which is sensitive and delicate, and when herbal soaps are used, you can be sure that the glycerine content is high absorbing water in the air and ensuring the skin remains soft and healthy (Buffoli, Maddalena *et al.*, 2018).

2.10 MEDICATED SOAPS AND THEIR BENEFITS

2.10.1 Dettol

Dettol Soap is a hygiene soap which provides trusted protection from a wide range of unseen germs. It cleanses and protects your skin for a hygienic, clean and refreshing feeling every day. Besides a germ protection formula, Dettol Skincare Soap also contains nourishing moisturizers that make your skin smooth and healthy. It contains nourishing multivitamins that cleanse your skin and provide protection against a wide range of germs. This medicated soap bar claims to provide 100% better protection than ordinary soaps. This antibacterial soap is quite gentle on the skin and works effectively to minimize skin dryness (Joloder, 2018). It is safe to use this antiseptic bath soap on acne-prone and sensitive skin types as well. For best results, take a bath with Dettol Skincare Soap daily to prevent skin infections. Regular use of this soap is recommended by the Indian Medical Association (Kelly, 2017). Dirt and bacteria can linger on your clothes and cause infections. Your daily personal hygiene involves cleaning and washing your

clothes as well. This is important if you have been ill or exposed to an ill person, or if you have been sweating (Kweon, 2018). Your clothes can be cleaned hygienically by adding Dettol Antiseptic Liquid or Dettol Disinfectant Multi-Use Hygiene Liquid to your laundry to kill germs. Your clothes will then smell clean and fresh when you wear them (Onanuga, 2018).

2.10.2 Tetmosol

Is a Grade 1 medicated skin-care soap, which is used in the treatment and prevention from scabies and other skin-related problems. It offers effective protection against skin issues, such as skin irritation, inflammation, rashes, and redness on the skin (Peari, 2015). Not only can it be used for your skin, but also works effectively in keeping your scalp and head free from body lice. It has a mild fragrance and can be used on a daily basis as well. It can ideally be used by adults and children alike (Fischetti, 2019).

Sulfiram (INN) or monosulfiram, trade name Tetmosol, is an ectoparasiticide used in the treatment and prevention of scabies. It is usually sold as a solution or medicated soap, sometimes in combination with benzyl benzoate (Michie, 2015).

2.10.3 Crusader

The crusader medicated soap helps eliminate clogged pores that can lead to infections, blemishes, pimples and body odours. This soap is for the face and body. You can also use this face as a mask before applying lightening cream.

2.11 INGREDIENTS USED IN MEDICATED SOAPS

All the popular brands such as Dettol, Dove, Himalaya Neem Soap etc are made up of strong detergents, harmful petroleum products, pesticides and harsh alcohol which are really toxic for you skin as if you use it regularly it will penetrate inside your body and produce harmful effects.

Dettol contains chloroxylenol BP in a 4.8 percent concentration of weight to volume, according to the UK Electronic Medicines Compendium. The product's other ingredients include isopropyl alcohol, pine oil, castor oil soap, caramel colouring and water. Chloroxylenol Kills Bacteria and Some Viruses Chloroxylenol is the active antibacterial ingredient in Dettol that kills bacteria, mycobacterium, fungi

and some viruses, according to an FDA report prepared for the American Cleaning Institute in 2014. Sodium Lauryl Sulphate, also known as SLS, is the ingredient added to soap to make it lather, and according to the EWG's Cosmetics Database, it's highly toxic. SLS carries a high concern for irritation of the skin, eyes, and lungs, as well as moderate concern for organ system toxicity. Sodium Palmate, Sodium Palm Kernelate, Aqua, Parfum, Talc, Sodium Chloride, Ecteronic Acid, Tetrasodium EDTA, Climbazole, Disodium Etidronate, Glycerine, CI 21100/CI 11680, CI 12490 are used in Dettol (Imarenezor, E. P. K *et al.*, 2020).

2.12 SIDE EFFECTS OF SOME CHEMICALS IN SOAP

Sodium hydroxide (caustic soda) causes eye irritation, burning sensation on skin, resulting permanent damage to the skin, causes dermatitis. Parabens causes skin irritations, swelling, redness, cellular level damage and has chance for skin cancer. CI 12490 (red pigment) causes skin burning and irritations. CI 11680 European scientific committee- harmful by inhalation, in contact with skin. Climbazole and tetrasodium EDTA causes skin rashes, itching, redness, irritation, inflammation on sensitive skin (Niranjan, Vora Rohan 2010).

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

To prepare medicated soap with enhanced anti-bacterial property against staphylococcus aureus.

3.2 OBJECTIVES

- To evaluate the antibacterial property of the different plant extract against *S. aureus*.
- To formulate the medicated soap with plant extract.
- To determine the anti-microbial property of prepared medicated soap with plant extract against *S. aureus*.
- To evaluate the properties of prepared medicated soap such as pH, TFM, foam forming ability and stability.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

Soap base (100% pure glycerine) was used for preparing soaps. Silicon mold was purchased commercially and used for pouring the soap. Sulphuric acid (concentrated), Methyl orange, Sodium chloride, Acetone, and Hexane were used in TFM test. Peptone broth and nutrient broth was used to grow culture for antimicrobial susceptibility test.

4.2 METHODS

4.2.1 Sample Collection

A. aspera, *A. indica*, *A. indica*, were collected from the village. These leaves were dried under the shade for a week and made as a fine powder.

Additionally, *S. auriculata* and *C. aromatica* are also dried under the shade for a week and made as a fine powder. These are added for aroma of the soap.



Fig: 4.1 Azadirachta indica



Fig: 4.1.1 Azadirachta indica powder



Fig: 4.2 Acalypha indica



Fig: 4.2.1 Acalypha indica powder



Fig: 4.3 *Achyranthes aspera*



Fig: 4.3.1 *Achyranthes aspera* powder



Fig: 4.4 *Senna auriculata*



Fig: 4.4.1 *Senna auriculata* powder



Fig: 4.5 *Curcuma aromatica*



Fig: 4.5.1 *Curcuma aromatica* powder

4.2.2 Soap Preparation

The soap is made under the melt and pour method. 20g of soap base which is made up of pure glycerin is chopped into small pieces and melted in the micro-oven. Natural ingredients are measured using a weighing scale and dissolved in distilled water. These ingredients are added to the melted soap base and mixed thoroughly and poured in the silicon mold. Leave the soap for 24 hours for completely set.



Sample – 1



Sample – 2



Sample – 3



Sample – 4



Sample – 5



Sample – 6



Sample – 7



Sample – 8



Sample – 9



Sample – 10



Sample – 11



Sample – 12



Sample – 13

Sample – 14

Sample – 15

Fig: 4.6: Different concentration soaps

4.3 ANTIMICROBIAL SUSCEPTIBILITY TEST

4.3.1 Isolation of microorganism

The isolated *S. aureus* plate was bought from Research & Development, Sathyabama Institute of Science and Technology. The subculture plate was prepared by inoculating the mother culture of *s. aureus* in the mannitol salt agar plates. Then the cultured plate was incubated at 37°C for 18-24 hours.



Fig: 4.7: Isolated bacteria culture

4.3.2 Preparation of peptone/nutrient broth

1.5 g of peptone was suspended in 100 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. A loop of culture was inoculated in 10 ml of peptone broth in a test tube, which was then incubated at 37°C for 24 hours.

1.3 g of nutrient broth was suspended in 100 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. A loop of culture was inoculated in 10 ml of nutrient broth in a test tube, and the test tube was incubated at 37°C for 24 hours.

4.3.3 Preparation of soap samples

A sterile blade was used to scrap one gram 1 g each of the soaps and which quantity was dissolved in 9 ml of sterile distilled water to give a stock solution of 10^{-1} . These stock solutions were then stored in a refrigerator in well-sealed containers for future use.

4.3.4 Preparation of Disks with Soap Samples

Disks of diameter 6 mm were bored using disc borer. The discs were then wrapped in foil paper and sterilized in a hot air oven at 100°C for 1 hour and were later soaked in the different soap solutions for a period of one hour to ensure full saturation of the soap preparations. The discs were then aseptically removed from soap solution and allowed to dry in an oven at 25°C. They were then packed into sterile bottles, corked and stored in the refrigerator for future use in susceptibility test.

4.3.5 Disk Agar Diffusion Method

The disk agar diffusion method as originally described by Bauer, (1966) was used. Mueller- Hinton agar plates were prepared for the isolates. Plates were dried with their lids ajar (slightly raised) at a temperature of 60°C in the oven for 15 minutes. 3.9 g of Mueller Hinton Agar was suspended in 100 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. The liquid was then poured into petri dishes and left to solidify.

The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates using sterile cotton swab. The plates were left for about 30 minutes; the disks were aseptically transferred directly into the sensitivity plates with the aid of a sterile forceps. Within 30 minutes of application, plates were inverted, incubated at 35°C for 24 hrs and then were examined for the zone of inhibition around the disk.

4.4 TOTAL FATTY MATTER (TFM)

4.4.1 Chemical preparation

4.4.1.1 Preparation of 50% sulphuric acid

25ml of distilled water were measured and poured into a beaker. Then, 25 ml of concentrated sulfuric acid was added to the beaker.

4.4.1.2 Preparation of saturated NaCl

38 g of NaCl crystals were dissolved in 100 ml of distilled water. The solution was vortexed and mixed thoroughly to ensure the NaCl dissolved properly, and then stored at ambient temperature.

4.4.1.3 Preparation methyl orange

For 0.1 g of methyl orange indicator powder was dissolved in 100 ml of distilled water. The solution was vortexed and mixed thoroughly to ensure proper dissolution, and then stored at ambient temperature.

4.4.2 Procedure for TFM

10 g of soap was placed in a conical flask. Then, 100 ml of distilled water was heated to 80°C and transferred into the flask containing the soap sample. The flask was swirled to mix the contents. The beaker was heated in a water bath to evaporate the hexane, and it was shaken while heating. Next, 5 ml of acetone was added to the beaker, which was then shaken and heated again as done previously. After 30 minutes, the beaker was removed from the water bath and placed in a hot air oven at 90°C for 1 hour. Afterward, the beaker was cooled for 10 minutes. Finally, the remaining extract settled at the bottom of the beaker was measured.

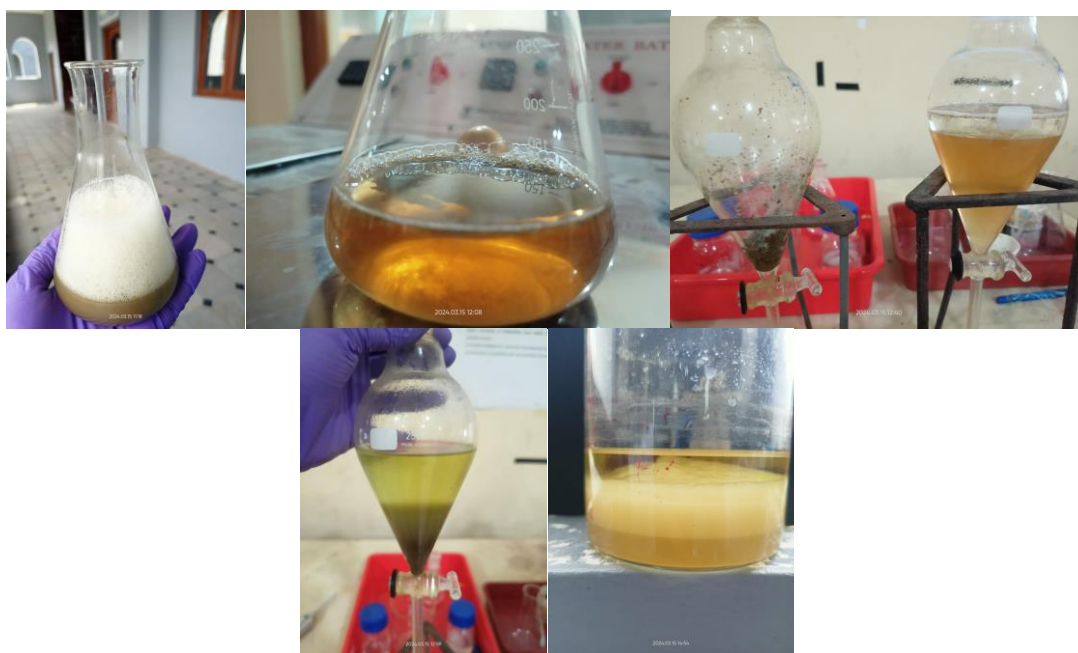


Fig: 4.8 TFM test

4.5 SKIN IRRITATION TEST (Hand Immersion Test)

Human volunteers immerse one of their hands in the solution of soap product to be tested while the other hand, simultaneously in a solution of a positive control for 10 min, twice daily for 4 consecutive days under supervision. Monitor the hand for any adverse skin responses prior to and after the last immersion. If the skin responses with the product tested are very similar to that manifested by the positive control, the product could be judged to have an unacceptable level of skin irritancy potential.

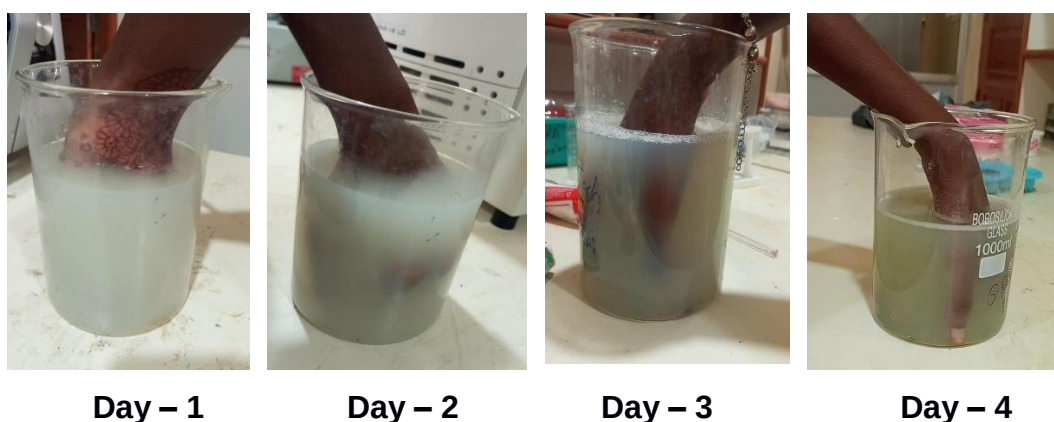


Fig: 4.9 Hand immersion test

4.6 PATCH TEST

A patch test is a skin test used to find the cause of a possible allergic reaction on the skin.

4.6.1 Procedure:

A small amount of soap is applied on the skin surface. Then it is left for 15-20 minute as shown in fig 4.10.



Fig: 4.10 Patch test

4.7 FOAM ABILITY AND STABILITY

The foaming capacity of soap depends upon the nature of the soap and its concentration. This may be compared by shaking equal volumes of solutions of different samples having the same concentration with same force for the same amount of time. The solutions are then allowed to stand when the foam produced during shaking disappears gradually.

4.7.1 Procedure

1 gram of soap is weighed and taken in a beaker. 20 ml of distilled water is added to dissolve the soap and mix well. 1ml of soap solution is taken in a test tube. Close the mouth of the test tube with the thumb and shake the test tube upside down for 10 times. Using a scale the foam is measured which is formed inside the test tube.



Fig: 4.11 Foam ability and stability

4.8 EXPERIMENTAL DESIGN

Statistical design of Box-Behnken experimental design was used to maximize the responses with precision of factor which has the largest effect on variables equidistant from the centre points. Leaf extract was taken in minimal,

middle and maximal level to analyse the best concentration. Three levels with three variables are taken and shown in Table 4.1 Fifteen samples were prepared as per Box-Behnken method with the process parameters are presented in Table 4.2

Table 4.1 Process parameters of Box-Behnken design

Variables	Process parameters	Levels		
		-1	0	+1
X1	<i>Achyranthes aspera</i>	4	6	8
X2	<i>Azadirachta indica</i>	3	4	5
X3	<i>Acalypha indica</i>	2	4	6

Table 4.2 Box-Behnken Design for the experiment

SI. NO	X ₁ (ml)	X ₂ (ml)	X ₃ (ml)	Soap ID
1	4	3	4	S1
2	4	5	4	S2
3	6	3	4	S3
4	8	3	4	S4
5	4	4	2	S5
6	4	4	6	S6
7	8	4	2	S7
8	8	4	6	S8
9	6	3	2	S9
10	6	3	6	S10
11	6	5	2	S11
12	6	2	6	S12
13	6	4	4	S13
14	6	4	4	S14
15	6	4	4	S15

For process optimization a three-level design was used by Box-Behnken design. A total of 15 experimental were performed to obtain the best concentration

of the soap against *S. aureus* as mentioned in the table 4.1, the concentration of *Achyranthes aspera*, *Azadirachta indica* and *Acalypha indica* are varied and the results are represented in the table 4.2 where “X1” represents, concentration of *Achyranthes aspera*, “X2” *Azadirachta indica*, “X3” *Acalypha indica*.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 ANTIMICROBIAL SUSCEPTIBILITY TEST

5.1.1 Zone of Inhibition for Prepared Soap Samples

Among the 15 samples, S9 shows the lowest zone of inhibition which has the concentration of different leaf extracts such as *Achyranthes aspera* 6ml, *Azadirachta indica* 3ml, and *Acalypha indica* 2ml.

The sample S2 and S10 with the different concentrations of leaf extracts such as *Achyranthes aspera* 4ml, *Azadirachta indica* 5ml, *Acalypha indica* 4ml and *Achyranthes aspera* 6ml, *Azadirachta indica* 3ml, *Acalypha indica* 6ml respectively, showed the highest zone of inhibition. In S9 the zone of inhibition was less due to the least concentration of *Azadirachta indica* and *Acalypha indica* compared to others.

Azadirachta indica and *Acalypha indica* acts powerfully against *S. aureus* than *Achyranthes aspera*. Though the *Achyranthes aspera* concentration is high but there is no increase in zone of inhibition.

Antiseptics soaps possess active germicidal chemical agent, it kills pathogen but its prolonged use should be avoided. As herbal soaps revealed similar antimicrobial activity like that of antiseptic soaps, they are more effective and considered better in our daily life. The soap is routine requirement of life, but it should have good active ingredients or plant extracted material which has the ability to kill bacteria but not to harm body tissues when used constantly (Varsha M Chaudhari 2016).

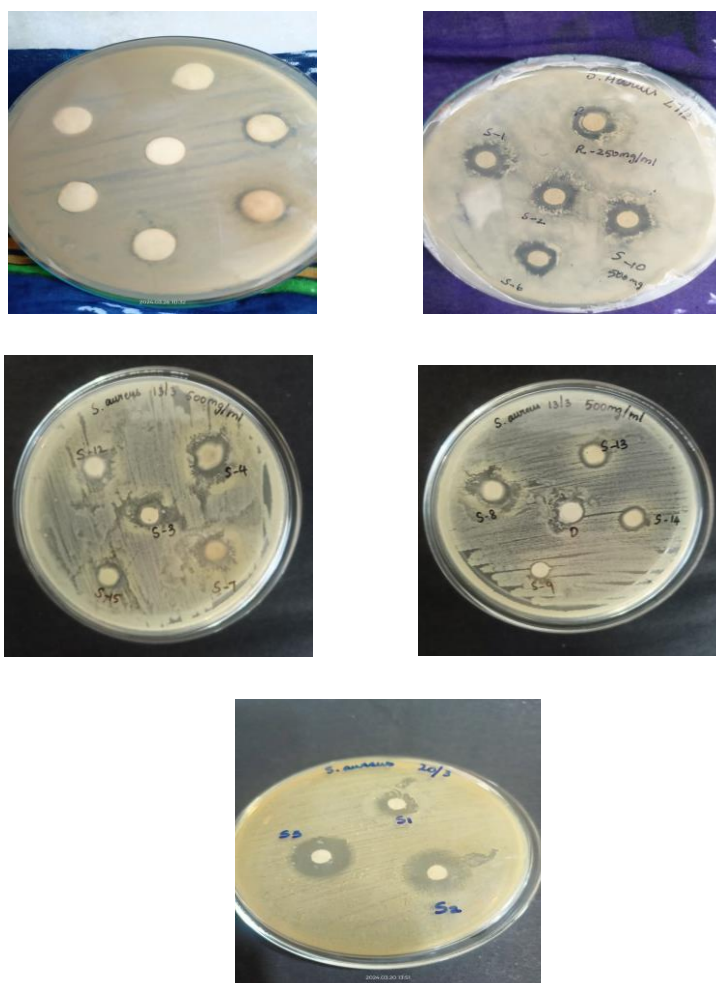


Fig: 5.1: Antimicrobial test for prepared soap with different concentrations

Table 5.1: zone of inhibition

SAMPLES	X ₁ (ml)	X ₂ (ml)	X ₃ (ml)	Zone of inhibition (mm)
S1	4	3	4	14
S2	4	5	4	18
S3	8	3	4	13
S4	8	5	4	14
S5	4	4	2	10
S6	4	4	6	15
S7	8	4	2	15
S8	8	4	6	14
S9	6	3	2	8
S10	6	3	6	18
S11	6	5	2	9
S12	6	2	6	11
S13	6	4	4	9
S14	6	4	4	9
S15	6	4	4	9

5.1.2 Final Equation in Terms of Actual Factors

Zone of inhibition = +39.25000 – 8.68750 *Achyranthes aspera* – 8.87500
Azadirachta indica + 6.00000 *Acalypha indica* – 0.375000 *Achyranthes aspera**
Azadirachta indica – 0.375000 *Achyranthes aspera** *Acalypha indica* – 1.000000
*Azadirachta indica** *Acalypha indica* + 0.968750 *Achyranthes aspera*² + 1.87500
*Azadirachta indica*² + 0.156250 *Acalypha indica*²

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to

determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the centre of the design space.

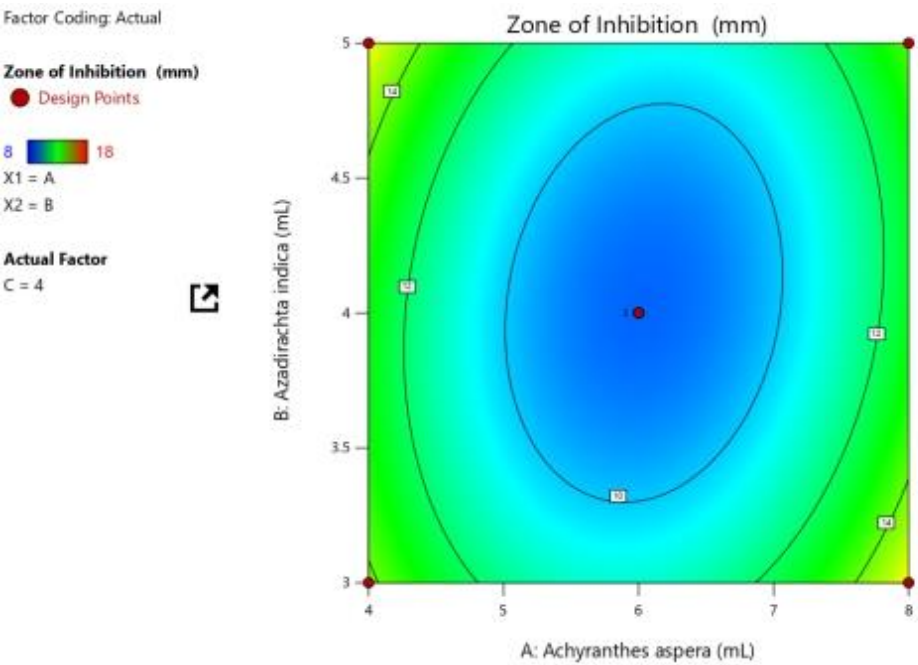


Fig: 5.2 Effect of *A. aspera* and *A. indica* concentration on zone of inhibition

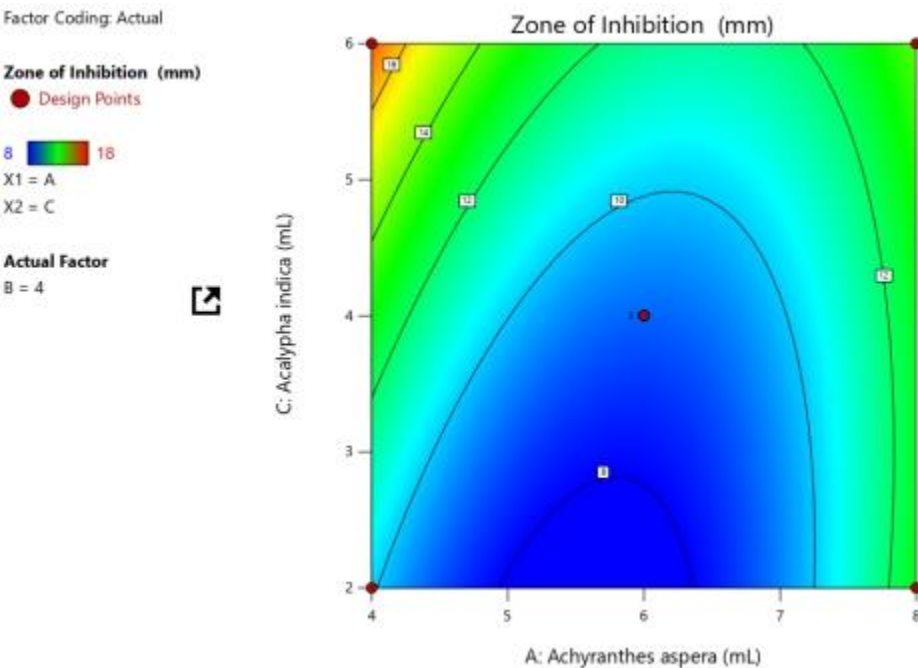


Fig: 5.3 Effect of *A. aspera* and *A. indica* concentration on zone of inhibition

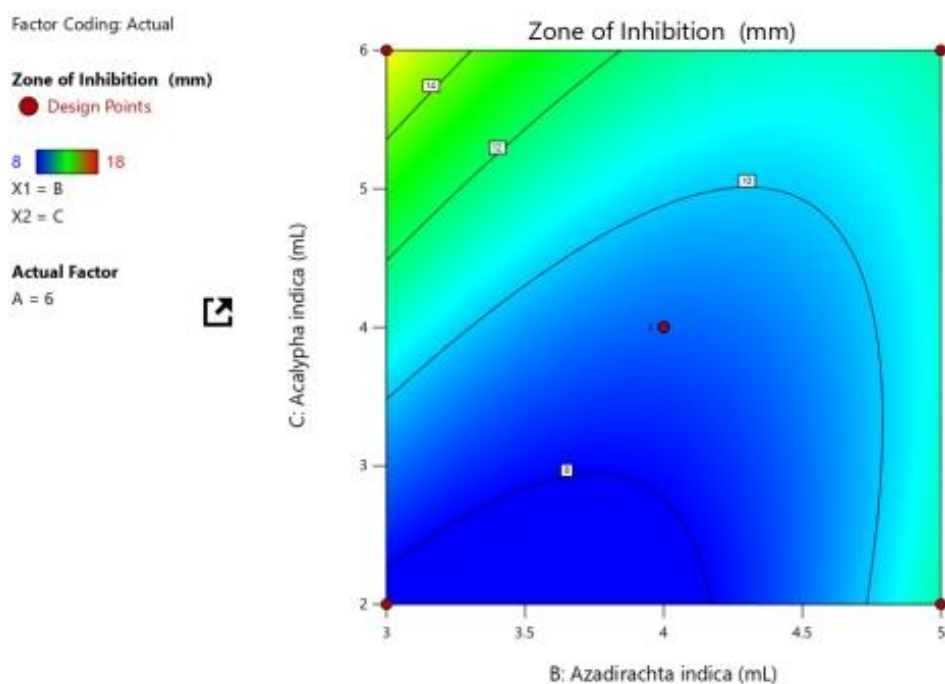


Fig: 5.4 Effect of *A. indica* and *A. indica* concentration on zone of inhibition

From the statistical analysis, it was found that *Acalypha indica* has a major effect on the formation of zone of inhibition.

5.2 pH TEST

pH 0 to 6 is acidic, pH 7 is neutral and pH 8 to 14 is basic. For handmade soap, pH testing is important to know whether the soap is ready to use on skin. Handmade soap is always alkaline (cleansing ability) with a safe range pH between 10 to 8 to use on skin. Anything above pH 11 is too harsh for skin and will cause irritations.

Normal healthy skin has potential of hydrogen (pH) range of 5.4-5.9 and a normal bacterial flora. Use of soap with high pH causes an increase in skin pH, which in turn causes an increase in dehydrative effect, irritability and alteration in bacterial flora. The majority of soaps available in the market do not disclose their pH. Majority of the soaps have a pH within the range of 7 – 10. Ayurvedic soaps, which claimed to be better than other soaps were not skin friendly in the aspect of their pH (Jose Tarun *et al.*, 2014).



Fig: 5.5 pH test

The pH of the prepared sample is 7 as shown in fig: 5.5

5.3 TOTAL FATTY MATTER (TFM)

Grade 1 soaps should have 76 per cent minimum TFM, while Grade 2 and Grade 3 must have 70 per cent and 60 per cent minimum TFM, respectively. The TFM content in a bathing bar should ideally be less than 60%, but not below 40%. Low TFM is usually associated with lower quality and hardness (Chaitali Nayak, 2023). The TFM of our soap is 67.69%.

5.3.1 TFM Calculation

$$\begin{aligned} \text{TFM} &= (\text{Initial weight} - \text{Final weight}) / (\text{sample weight}) \times 100 \\ &= (10 - 1.2) / 13 \times 100 \\ &= 67.69\% \end{aligned}$$



Fig: 5.6: Total fatty acid test

5.4 SKIN IRRITATION TEST

Hand immersion is a skin test used to check whether any changes occurring in hands after immersing the hand in soap solution. In our product there is no changes was observed. Monitor the hands for any adverse skin responses prior to and after each immersion and finally on the morning after the last immersion. If the skin responses with the product tested are very similar to that manifested by the positive control, the product could be judged to have an unacceptable level of skin irritancy potential (Faoagali JL *et al.*, 2002).



After immersion

Fig: 5.7: Hand Immersion Test

There was no changes or irritations while immersing the hand for continuous 4 days as shown in fig 5.7

5.5 PATCH TEST

A patch test is a skin test used to find the cause of a possible allergic reaction on the skin. In our product there was no allergic reaction on the skin.

Patch tests are tools used in the identification of the etiologic agent of allergic contact dermatitis. The product which has acidic cause irritations on the skin (Rosana Lazzarini *et al.*, 2013).



Fig: 5.8: Patch test

No irritations were observed shown in fig 5.8

5.6 FOAM ABILITY TEST

Foam test is done to check whether the soap has the capability of producing foam (Krishnamoorthy C 2015).

The results of the foam ability test for herbal soap and other commercially available soaps are shown in the fig 5.9

5.6.1 Foam height

Sample A (Herbal soap) – 4.3 cm, Sample B (Dettol) – 4 cm, Sample C (Savlon) – 4.1 cm and Sample D (Life buoy) – 4.5 cm.

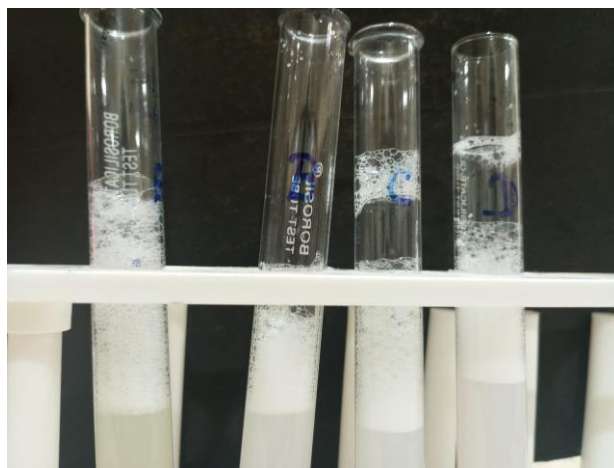


Fig: 5.9: Foam ability test

CHAPTER 6

SUMMARY AND CONCLUSION

The soaps are cleaning agents routinely used for cleaning purposes and removing germs. Soaps and detergents disrupt the microbial cell membrane and disrupt cells proteins. Soaps tested in the present research work showed varied levels of activity against the *S. aureus*. However, prolonged use of these soaps could lead to development of microbial resistance in future. Hence, our product among others that showed anti-bacterial activity can be used to prevent skin infections. The main aim of this study was to identify the antimicrobial activity of antiseptic soaps and herbal soaps. Antiseptics soaps possess active germicidal chemical agent, it kills pathogen but its prolonged use should be avoided. As herbal soaps revealed similar antimicrobial activity like that of antiseptic soaps, they are more effective and considered better in our daily life. The soap is routine requirement of life, but it should have good active ingredients or plant extracted material which has the ability to kill bacteria but not to harm body tissues when used constantly. *Achyranthes aspera* has less effect against *S. aureus* compared to *Azadirachta indica* and *Acalypha indica*, as for people who need further research, they can replace *Achyranthes aspera* by another higher effective leaf extract to make a soap against *S. aureus*

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