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Formulation And Evaluation Of Novel Herbal Anti- Dandruff Shampoo With Pomegranate Peel Extract

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Abstract*:* Dandruff is a scalp disorder common to human population. Many of the studies shows that the presence of *Malassezia* species cause dandruff but according to recent studies it is proved that bacteria has a larger role in causing dandruff, it is also revealed that bacteria had a stronger relationship with the severity of dandruff than fungi. Herbal shampoo consists of natural composition that avoids harsh chemicals making it gentle on the scalp and hair. The goal of this study is to formulate a herbal shampoo with pomegranate peel extract. Pomegranate peel extract is selected for its well-known antimicrobial properties on *Staphylococcus epidermidis* which is also proven to be a causative agent of dandruff. Based on UV Spectra Punicalagin, Punicalin, Ellagic acid were detected which have antibacterial property against the said bacteria, while flaxseed gel was chosen as a base for its shear thinning and thickening effects. The formulation process is focused on optimizing the concentration of the pomegranate peel extract, in this study we made three different formulations with increasing concentrations of Pomegranate Peel Extract (PPE), F1, F2, F3. Various parameters like physical parameters, foaming studies, cleaning action, wetting time, surface tension, percentage solid content are evaluated. The shampoo's anti-bacterial activity was evaluated, the Minimum Inhibitory Concentration (MIC) was measured and compared with Clindamycin as standard using the agar well diffusion method. 200ul of the shampoo was found to have better zone of inhibition than Clindamycin 32mg/ml.

The F-3 was found to have had lesser wetting time and better detergency compared to F-1 and 2. The solid content was 22.85 which was higher than F1 and F2 but is within the specifications. It decreased the surface tension better than F1 and F2. The pH was under acceptable range according to the skin pH.

Keywords: Pomegranate peel, Flax seed, Anti-dandruff, Staphylococcus epidermidis, Ellagitannins, Vetiver roots

Abbreviations: Pomegranate Peel Extract (PPE), Flax seed Protein (FP's), Flaxseed Gum (FG), Ultrasound Assisted Extraction (UAE), Ultraviolet visible spectroscopy (UV-VIS)

I. INTRODUCTION

1.1 Dandruff and its causes

Dandruff- Pityriasis capitis, also known as pityriasis simplex capitis, is a prevalent, chronic scalp ailment that has been known since ancient times and affects over half of the world's population. Dandruff, which typically appears around puberty or after, can be a stressful ailment because of its obvious appearance, recurrence, and chronic nature, all of which can lead to psychosocial and social issues for the affected person. Flaking, pruritus, and mild inflammation are the main symptoms of this scalp-specific ailment, which can be linked to either oily (sebum-producing) or dry scalp type of skin. Dandruff may appear under a microscope as a flakes of pale-yellow skin cells. (1)

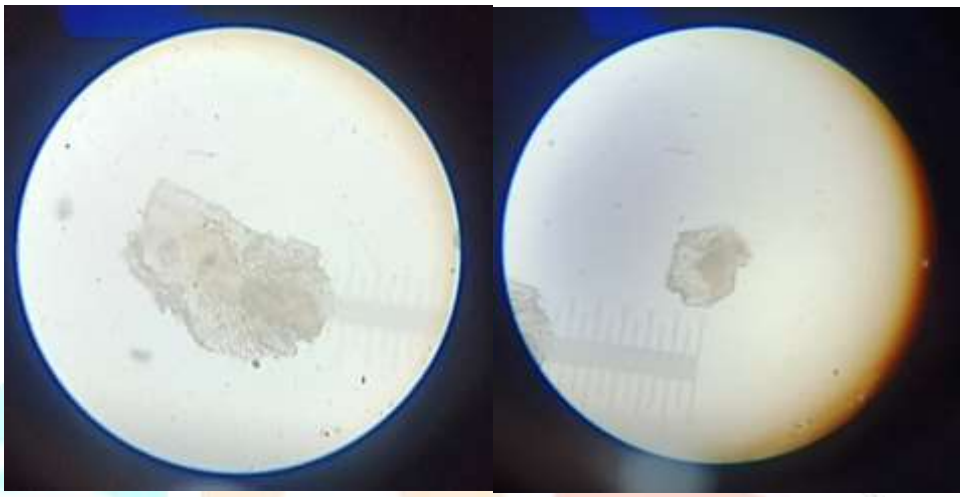

Figure 1: Dandruff under microscope

Many hypothesis on the development of dandruff have been widely recognized based on decades of research into the phenomenon.
According to Dawson and others, sebum secretion, *Malassezia* fungus, and individual susceptibility are the causes of dandruff.

- Lipophilic yeasts called *Malassezia* are primarily present on seborrheic areas of the body. Research has shown that people with dandruff had larger concentrations of *Malassezia* yeast especially *Malassezia globosa* and *Malassezia restricta* on their scalps, which is correlated with the development and intensity of seborrheic dermatitis.
- According to Gaitanis, dandruff is caused by certain tryptophan metabolic products made by *Malassezia*, like indole derivatives. Human sebum triglycerides are hydrolysed by *Malassezia*, which produces unsaturated fatty acids including arachidonic and oleic acid, which can result in anomalies of the stratum corneum such as, intracellular lipid droplets and uneven corneocyte envelope due to improper keratinocyte development.
- These alterations cause the epidermal barrier to malfunction and set off an inflammatory reaction, which may or may not manifest as localized inflammation. Furthermore, pro-inflammatory cytokines such IL-6, IL-8, and TNF-α are produced by keratinocytes as a result of these metabolites, which prolongs the inflammatory response. Moreover, prostaglandins, a class of pro-inflammatory mediators that can trigger inflammation by neutrophil recruitment and vasodilation, can be generated from arachidonic acid. These elements support the growth of basal layer epidermal keratinocytes (KCs), which in effect drives up the creation and shedding of corneocytes (held together by an intercellular adhesion known as corneo-desmosomes), which is clinically characterized as dandruff. More research has recently shown that dandruff is related to the disequilibrium in the percentage of the main bacterial and fungal populations. (2)

Studies on dandruff have long mostly examined fungi, specifically the species of *Malassezia*. But the human scalp is also home to a different bacterial microbe community that consists of both aerobic and a facultative anaerobic bacterium, which include *Staphylococcus* and *Propionibacterium acnes.* Eleven bacterial phyla were identified using 454 pyrosequencing of the scalp dandruff microbiome; nevertheless, the majority of sequences were attributed to Actinobacteria (64.9%) and Firmicutes (32.5%). The Actinobacteria *Propionibacterium*

(63.3%) and the *Firmicutes Staphylococcus* (32.4%) accounted for over 95% of all sequences. P. acne accounted for 99.7% of the *Propionibacterium* and 94.9% of the *Staphylococcus* species including *S. epidermidis, S. capitis and S. caprae.*

It was discovered after multiple investigations that there was no tight relationship between the fungi in species and the bacteria in genus (no relation between bacteria and fungi that cause dandruff). Additionally, there was a higher correlation between bacteria and dandruff than there was between fungi & dandruff. (3)The reasons of dandruff are frequently complex and connected to one another. The following are the factors that received the most attention:

(a) An excessive amount of yeast, Malassezia species over colonization.
(b)Bacterial species imbalance.
(c) Impaired function of the epidermal barrier.
(d) Elevated fatty acid metabolites generated from sebum and squalene peroxidation, a component of sebum.
(e) Perivascular leukocyte infiltration; these factors can all cause a modest inflammatory response and immunological response, either separately or in combination. (1)

Our shampoo as a part of a novel approach aims to eliminate the microbial cause of dandruff, as according to the literature there was a stronger relationship between bacteria and dandruff than fungi and dandruff. Our shampoo aims to target and decrease the concentration of *Staph. Epidermidis* causing the decrease or control of the dandruff.

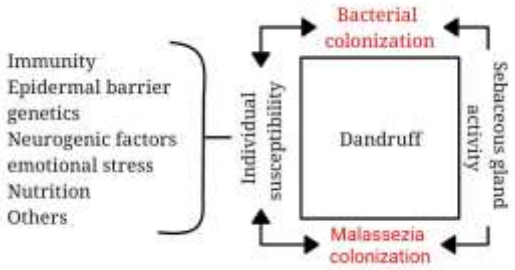

Figure 2: Predisposing factors and their interactions in the pathogenesis of dandruff

1.2 Shampoo

1.2.1 What is shampoo?

Shampoo is a hair care item that cleans the skin and scalp in addition to the hair. It also makes hair seem nicer and easier to manage by eliminating many types of filth, such as perspiration, sebum and environmental debris. The Hindi word "shampoo," which means "to press or massage," is where the English word "shampoo" originated. It was used to refer to washing by massaging the skin and hair. It is no longer advised to clean hair using traditional soaps that were once used for both the skin and the scalp since they don't lather well and leave behind "soap scum" that is difficult to rinse out when combined with hard water. (4)

1.2.2 Types of shampoos

Table 1: Types of shampoo

Normal shampoo	Regular shampoos are made to clean one's hair of those without chemically treated hair and with modest sebum production. These shampoos use minimal conditioning and sodium or ammonium lauryl sulfate for good washing.
Oily hair shampoo	Shampoo for oily hair is made to rid the hair and scalp of extra sebum. Strong surfactants, like lauryl sulfates, can be used for this purpose with little to no conditioner. Because these shampoos contain higher surfactants, they are more abrasive on hair than regular hair shampoos. Oily hair does not require conditioning because of its excessive sebum production.
Dry hair shampoo	Dry shampoo for hair use good conditioning techniques along with mild surfactants, like sulfosuccinates, to provide gentle cleansing. Dry hair and scalp require more care because they produce less sebum.
Every day shampoos	Are designed to be gentle formulas that may be applied daily without leaving an excessive amount of oil residue or drying out the hair.
Deep cleansing shampoos	Are made to provide the hair a deep clean. Typically, these products are used to remove mousse, sprays, and gels that are kept in hair. including greasy hair shampoos, these shampoos use stronger surfactants, including sodium or ammonium lauryl sulfate, to effectively remove grime
Baby shampoos	are typically softer because they contain amphoteric surfactants like betaines. They have low sebum production and are non-irritating.
Gray hair shampoos	Consists of items that have blue dyes in them to give gray hair a more vibrant, less yellowish tone. Excessive dosage or frequent use may give the hair a bluish tint.
Hair dyeing shampoos	Are unique mixtures intended for usage following permanent hair coloring. These shampoos feature an acidic pH and cationic surfactants, which neutralize any remaining alkalinity from the hair coloring chemicals and reduce cuticle swelling.
Medicated shampoo	Are made to provide the hair and scalp with additional advantages beyond just cleaning and conditioning. The majority of medicated shampoos have active

	chemicals that reduce scaling and irritation.
Dry shampoos	Were the first categories of goods for cleaning hair. The term "dry" in their name does not relate to the kind of hair that they should be used on; rather, it refers to the dosage form (powder or powder-based aerosol). Powders that are effective at absorbing oil, like as talc, silica, magnesium stearate, kaolin, and starch, are found in dry shampoos. (4)

1.2.3 Anti-dandruff shampoo

The goal of anti-dandruff products is to lessen the development of dandruff flakes. Several "active ingredients" that have antibacterial or anti-mitotic properties are used in the treatment process. Any type of water will work, but an effective shampoo should produce a lot of foam almost instantly, regardless of the kind of dirt or fat that needs to be taken out of hair. Despite the fact that foam generation has no bearing on or proportionality to the cleansing function, consumers will always psychologically choose high foam products. Dandruff can only be adequately managed and controlled; it cannot be completely eliminated. (5, 6)

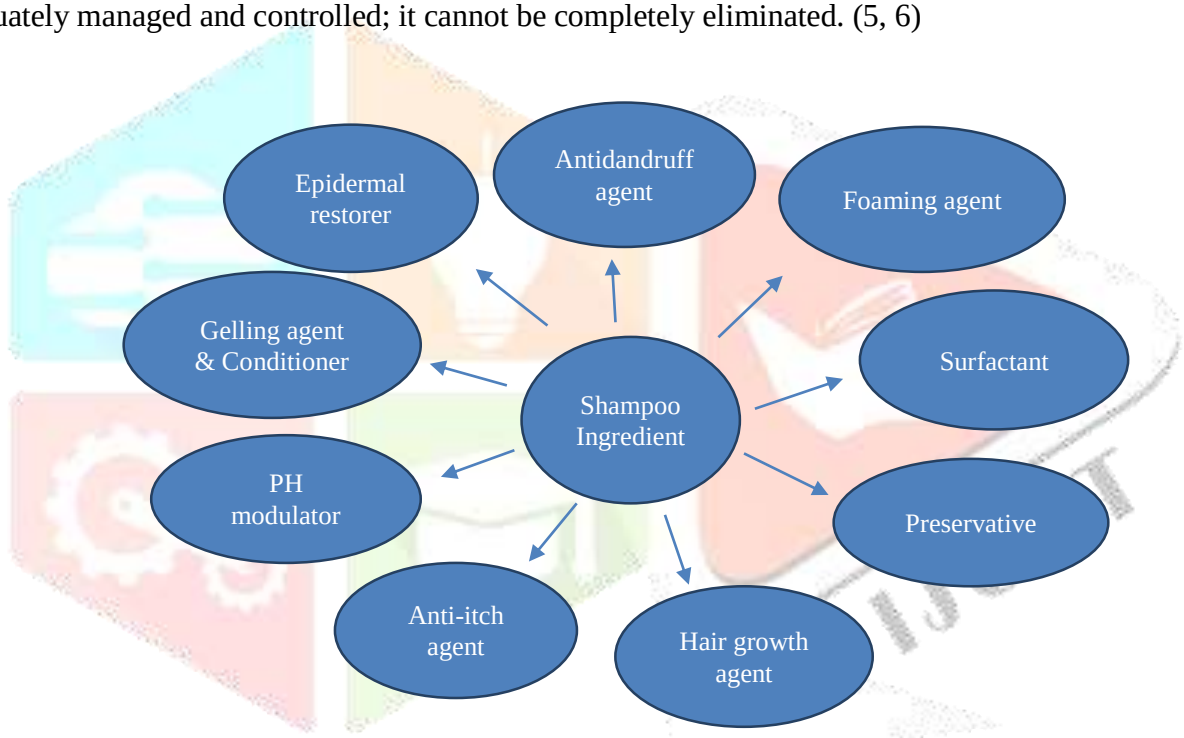

Figure 3: Ingredients in our anti-dandruff shampoo

1.3 Natural vs chemical Agents

Although they might not create as much foam, natural surfactants remove debris, oil, and other contaminants from the hair in an efficient manner. Conversely, synthetic surfactants tend to be quite successful at removing oil and grime from hair because of their great foaming ability. But the ability to cleanse is not always correlated with foam formation. In addition, natural surfactants are kinder to the hair and scalp than synthetic ones. They work better with sensitive skin because they are less prone to irritate and dry out the skin. Furthermore, natural surfactants preserve the hair's natural moisture balance and are less likely to make hair frizz and dry. Conversely, synthetic surfactants have the potential to be harsh and deplete the hair's natural oils, which can result in dry hair. In this way synthetic agents can be delirious to health and show many adverse effects. (7)

Just like how artificial surfactants cause adverse effects to hair, many artificial anti-dandruff agents cause side effects to human health which can range from a simple itch to headaches and jaundice. Some of these effects are mentioned below.

Table 2: Side effects of artificial anti-fungal agents

S.no	Side effects	Drugs
1	Non-melanoma skin cancer prolonged therapy	Voriconazole
2	Fever, chills	Ketoconazol Voriconazole Flucytosine
3	Rash	Flucytosine Ketoconazole Voriconazole
4	Nausea, vomiting	Fluconazole Ketoconazole
5	Abdominal pain	Ketoconazole Voriconazole
6	Amemia	Amphotericin B Flucytosine
7	Leukopenia, thrombocytopenia	Flucytosine Fluconazole
8	Decreased renal function	Amphotericin B Voriconazole
9	Headache	Ketoconazole Caspofungin
10	Dark urine, clay-colored stools, jaundice	Anidulafungin C, micafungin (7)

II. LITERATURE REVIEW

Evania Arneta et al. (2023) Alkaloids, flavonoids, saponins, and phenolics found in pomegranate peel extract exhibit antibacterial action against *Staphylococcus aureus* and *epidermidis*, with a Minimum Inhibitory Concentration (MIC) of 1.9 mg/ml. (9)

Yaxian Mo et al (2022) Pomegranate peel contains a variety of structural tannin forms that are largely hydrolyzable and water-soluble phenolics. These substances can be categorized into four primary classes according to their structural traits: complex tannins, condensed tannins, ellagitannins, and gallotannins. Tannins can also have antimicrobial effects by depleting metal ions, precipitating membrane proteins, and inhibiting the function of certain enzymes. (11)

Zhijue Xu et al (2016) A widespread scalp condition that has been around for ages, dandruff affects around 50% of people globally. Despite decades of research on the subject, no coincident theory has gained widespread acceptance. According to Dawson and other theories, sebum secretion, *Malassezia* fungus, and individual sensitivity all contribute to dandruff. According to Gaitanis, dandruff is brought on by certain tryptophan metabolic products made by *Malassezia*, including indole derivatives. Another study has shown that dandruff is related to the disequilibrium in the percentage of the main bacterial and fungal populations. Research on dandruff has mostly concentrated on fungus, namely the species of *Malassezia*, which are important fungi that colonize human scalps and are the primary constituents of the cutaneous fungal microbiome.

The two most clinically relevant *Malassezia* species of the 14 known species that have been cultured are *M. restricta* and *M. globosa*. Skin conditions like dandruff, seborrheic dermatitis, pityriasis dermatitis, and atopic dermatitis have all been linked to these species, according to reports.

But the human scalp is also home to a different bacterial microbe community that consists of both aerobic and facultative anaerobic bacteria, such as Staphylococcus and P. acnes. (3)

Borda LJ et al (2015) Dandruff and seborrheic dermatitis (SD) are two different manifestations of exactly the same condition that affect the body's seborrheic regions. Only affecting the scalp, dandruff is characterized by flaky, itchy skin that is not observably inflamed. In addition to affecting other seborrheic areas, SD can cause pruritus, irritation, and itchy, flaking, or scaling skin. The pathophysiology of SD and dandruff is influenced by a number of intrinsic and external factors, including sebum secretions, fungal colonization on the skin's surface, individual sensitivity, and interactions among these factors. They have compiled the most recent information on dandruff covering predisposing factors, clinical manifestations and diagnosis, treatment, epidemiology, severity of disease, and genetic investigations in people and animal models. In order to effectively treat SD and dandruff, symptoms must be eliminated using antifungal and anti-inflammatory medications, related symptoms like pruritus must be lessened, and overall scalp and skin health must be maintained to support remission. (2)

Baki G et al (2015) The goal of anti-dandruff products is to lessen the development of dandruff flakes. Several "actives" that have antibacterial or anti-mitotic properties are used in the treatment process. Any type of water will work, but a high-quality shampoo should produce a lot of foam almost instantly, regardless of the kind of dirt or fat that needs to be taken away from hair. Despite the fact that foam generation has no bearing on or proportionality to the cleansing function, consumers will always psychologically choose high foam products. Dandruff can only be adequately managed and controlled; it cannot be completely eliminated. (5)

Howell AB et al. (2013) Using the disc diffusion method, the effects of three different doses of a methanolic pomegranate husk extract—4 mg/mL, 8 mg/mL, and 12 mg/mL—on the proliferation of dental bacteria were examined. The pomegranate extract exhibited antimicrobial properties against S. aureus and S. epidermidis at all doses. (8)

III. AIMS AND OBJECTIVES

The aim of this study involves evaluating the efficacy of Pomegranate peel extract as anti-dandruff agent and flaxseeds gel as gelling and entrapping agent in novel herbal shampoo.

- To evaluate the antidandruff efficacy of pomegranate peel extract shampoo by assessing its anti-bacterial action on *Staphylococcus epidermidis.*
- To prepare herbal shampoo with Pomegranate peel extract as anti-dandruff agent and flaxseed mucilage/gum as gelling agent.
- To evaluate cleansing, wetting and detergency effects of herbal shampoo.

IV. MATERIALS AND METHODS

4.1 Plant materials and reagents

For this study, the combination of Soapnut extract, Shikakai extract, Vetiver root extract, Ginger oil, Tea tree oil and Rosemary extract were used which were purchased from BRM Chemicals, a recognised company with Certificate Of Analysis (COA) for all the mentioned ingredients. Flaxseeds were purchased from a local vendor in Hyderabad. Pomegranate peel powder was purchased from Wonder herbals.

The following reagents were used, Molisch reagent from virat labs, Hagers reagent and Ethanol from Research Lab, Benedict's reagent from SDFCL, 1% Ferric chloride solution and lead acetate from Molychem, and Sodium hydroxide from Finar.

SN	INGREDIENT	BIOLOGICAL SOURCE	USE OF INGREDIENT
1.	Pomegranate peel powder	Dried powder of Punic granatum Family: Punicaceae	Anti dandruff agent
2.	Reetha extract	Fruit of Sapindus mukorossi Family: Sapindaceae	Foaming agent
3.	Shikakai extract	Fruit of Acacia concinna Family: Leguminosae	Surfactant
4.	Rosemary extract	Flowering tops of leafy twigs of Rosmarinus officinalis Family: Lamiaceae.	Preservative
5.	Tea tree oil	Leaves of Melaleuca alternifolia Family: Myrtaceae	Hair growth
6.	Ginger oil	Rhizomes of Zingiber officinale Family: zingiberaceae	Anti- Itching agent
7.	Flax seed gel	Dried ripe seed of Linum usitatissimum Family: Linaceae	Gelling agent and conditioner
8.	Vetiver root extract	Dried roots of Chrysopogon zizanioides Family: Graminae/Poaceae	Restores skin and increase reconstruction of human epidermis and scalp

Table 3: Ingredients and their uses

4.2 Summary of Ingredients

4.2.1 Pomegranate peel

The *Punicagranatum* L. genus, *Punicaceae* family, includes the pomegranate, which has its origins in Iran, India, China, and the Mediterranean region. The husk, juice, and seeds make up the three components of the pomegranate fruit. Its various parts have been used as a diarrhoea and dysentery treatment, an anti-helmintic especially against tapeworms, an astringent, a hemostat, and a remedy for diabetes.

Pomegranate peels are a good source of bioactive substances like dietary fiber, vitamins, minerals, and polyphenols. Pomegranate peels' bioactive components can be used as functional ingredients to make greater use of the by-product resources, thereby contributing. The hexose monophosphate shunt pathway is used in the production of glycosides and polysaccharides. Phenols, tannins, and aromatic alkaloids all use the shikimate pathway. For the synthesis of alkaloids and phenols acetate–malonate pathway, while the mevalonate route is considered for steroids and terpenes.

The categories of active components are-

Polyphenols

The total phenolic content of pomegranate peel varied depending on the species, extraction techniques, and extraction solvents used. Among these, tannins, flavonoids, and phenolic acids are the primary phenolic compounds.

Tannins

These substances can be categorized into four primary classes according to their structural traits: complex tannins, condensed tannins, ellagitannins, and gallotannins. Punicalagin, a kind of ellagitannin, is the primary component of pomegranate peel tannins and the material that gives pomegranate peels their distinctive flavor; its amount is significantly higher than that of other fractions. Through endogenous endo-esterification breakdown of the hexahydroxybenzoic acid molecule, punicalagin can yield ellagic acid.

The pomegranate's antibacterial effect is attributed to ellagitannin, punicalagin, punicalin, ellagic acid, and its derivatives. After thereafter, ellagic acid can polymerize with sugar ligands to create ellagitannins with intricate structures. Tannins work against bacteria by depleting metal ions, precipitating membrane proteins,

and inhibiting enzyme function. (Howell et al., 2023) found that Staphylococcus epidermidis growth was decreased by pomegranate peel extract at all concentrations higher than 4 mg/ml. The present study is mainly concerned with the extraction of tannins, as they possess the desired activity for our shampoo. (10)

Flavanoids

The term "flavonoids" primarily describes a group of substances that are produced from flavanone (2-phenylchromanone), which is present in large amounts in pomegranate skins. The ring's various substitution patterns give rise to a number of distinct subclasses, including anthocyanidins, proanthocyanidins, flavonoids, and flavonols. Flavonoids, also known as phytoestrogens, are known for their strong antioxidant action and may lower the risk of hormone-related malignancies.

Phenolic acids

Pomegranate peels have been found to contain phenolic acids, such as gallic, ellagic, caffeic, butyric, erucic, ferulic, and cinnamic acids. Pomegranate phenolic acid profiles and concentrations differ based on the region in which they are planted. Through the transmembrane diffusion of phenolic compounds, they exhibit antibacterial action that can lead to cell death and cytoplasmic acidification. (12)

4.2.2 Flax seed gum

Flax seed, which is a traditional crop grown for oilseeds or fiber, is a member of the *Linum usitatissimum L.* genus in the *Linaceae* family. There are many compounds with hydrocolloidal qualities found in flaxseed. Hydrocolloids, primarily polysaccharides and proteins, are colloidal materials that have a preference for water. Hydrocolloids thicken and stabilize formulations by forming thick and viscous solutions, pseudo-gels, or gels in water. (13)

Two more important natural hydrocolloids found in flaxseed are Flaxseed Gum/ Flaxseed Gel (FG) and Flaxseed Proteins (FPs). Made up of neutral and acidic monosaccharides, FG is a hetero-polysaccharide. Because it accumulates in the seed coat, water may readily extract it from flaxseed hulls, meals, or whole seeds. FG, sometimes referred to as flaxseed mucilage, is found in the outermost layer of the seed coat and ranges in content from 3.5% to 15.0% throughout the entire seed. Conlinin makes up the majority of the protein in FG. With water binding values of 16–30 g water/1g FG, FG has a considerable water-binding capacity. Shear-thinning flow behavior of FG is exhibited by a higher fraction of neutral polysaccharides (arabinoxylans), that possess a greater MW and enhance viscosity.

The pH of FG also has an impact on viscosity and fluidity. At pH 2, FG's lowest viscosity is visible. The viscosity rises with increasing pH until it reaches pH 8, at which point it is three times greater than FG at pH 2. But viscosity drops once again with pH values greater than 8. (14)

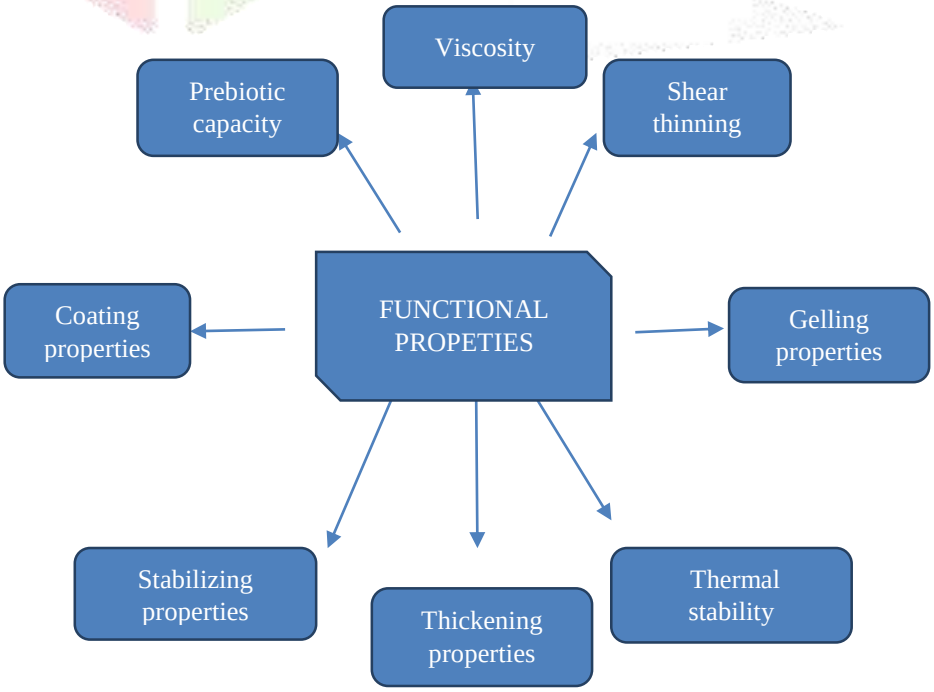

Figure 4: Properties of flax seed gel (14)

4.2.3 Soapnut

The tropical tree *Sapindus mukorossi Gaertn*. belongs to the *Sapindaceae* family. Saponins have foam-forming characteristics and lower the surface tension of water in aqueous solutions. The amphiphilic structure of saponins, which comprises of hydrophilic sugar groups (glycone) and a hydrophobic skeleton called aglycone (or genin), gives them their detergent qualities. The structural diversion of saponins in nature is based on the two glycoside-forming components. The triterpenoid saponins, which are found in several plant sections and induce fruits, galls, or roots, are the most significant components of the plant. The pericarp contains a significant amount of saponins, making up approximately 10.1–11.5% of the fruit; in the drupe, this percentage rises to 56.5%. Additionally, 10% of the fruit is made up of mucilage, sugars, and sesquiterpene oligo-glycosides.

Because they are widely available in nature, surfactants generated from plants are seen to be beneficial natural sources. They are also less expensive, biodegradable, energy-efficient, and environmentally beneficial. Saponin is one of the several types of naturally occurring surfactants that come from plant sources. Of all the bioactive chemical substances, plant saponins possess the greatest number of surfactant characteristics.

They work as non-ionic surfactants, solubilizing the oils and debris from hair strands and scalp while lowering the surface tension of water and interfacial tensions. The shampoo's cleaning power increases with decreasing surface tension. Since glycosides are hydrolyzed, saponins are regarded as somewhat acidic substances. At 8 g/L, the pH values of the Sp. mukorossi and Sp. trifoliatus extracts are 4.6 and 4.8 in distilled water, respectively. The nut extracts have good surface activity and moderate wetting capabilities, according to the results. Hydrophilic saponins are identified for both the extract and the pure saponin, with HLB values of 16.8 and 18.7, respectively. The CMC is $7.50 \times 10{-}3$ g/cc (15,16,17)

4.2.4 Shikakai

The *Fabaceae* family includes the climbing shrub *Acacia concinna* (Shikakai). Asia is home to many cultivators of this plant, particularly in central and southern Asia. Traditionally, shikakai's pods have been utilized as a herbal detergent to clean hair. The pods contain a lot of saponins, which are organic cleaners. Acacia acid triglycosides, or saponins, are composed of different kinds of saccharine derivatives. These triglycosides are made up of glucose, arabinose, xylose, and other glycons or sugar moieties that are connected to the acacia acid moiety via oxygen. Shikakai is regarded as a good foaming component and a decent alternative to chemical foaming agents because of its high saponin concentration. The CMC is $7.00 \times 10{-}2$ g/cc. (18)

4.2.5 Vetiver root extract

Tropical climates are home to a large cultivation of Vetiver, or *Chrysopogon zizanioides*, a perennial grass. According to a study, the chemical components include syringaldehyde, compound X, oplopanone, isovalencenic acid, zizanoic acid, compound Y, compound Z, compound W, teuhetenone compound Y, and minor compounds vanillin, valencene-11,12-diol, solanerianone A, and compound -V. A mixture of glucose and fructose was found in the most polar fractions.With the exception of compounds V, W, Y, and Z, which have never been described before, and compound X, which was first reported as a natural compound, the main compounds found in this exhausted root extract of Chrysopogon zizanioides are well-known sesquiterpenic constituents of Vetiver essential oil.

Researchers have shown that while vetiver extract stimulated human sebocyte cell lines to produce sebum, it also enhanced the quality of the sebum by producing certain antimicrobial lipids. Second, using Reconstructed Human Epidermis and skin explants, we showed that Vetiver extract could re-establish the skin barrier by increasing the neo-synthesis of skin lipids. Additionally, it was demonstrated that the lipid transport and epidermal cornification were increased by the vetiver extract. It had a notable impact on adipocyte maturation and adipogenesis. All of these actions were validated by evidence that the vetiver extract increased hydration and enhanced sebum production by changing the amount and structure of lipids. Vetiver extract worked extensively on adipose tissue to reduce skin tiredness and provide a plumping impact. (19)

4.2.6 Ginger oil

Numerous bioactive components, such as phenolic molecules, terpenes, lipids, and carbohydrates, have been found to be present in ginger. Therefore, phenolic chemicals and terpenes are primarily responsible for its pharmacological activities. Four phenolic compounds—gingerols, shogaols, paradols, and zingerone—among the 400 types of chemicals found in ginger are primarily responsible for its biological benefits. Generally, in vitro and in vivo investigations have established their high anti-inflammatory and antioxidant activity. The

structure and characteristics of ginger's four primary phenolic components are outlined in Figure 2. The main polyphenols in fresh ginger are gingerols, specifically 6-, 8-, and 10-gingerol. Gingerols can be converted into matching shogaols by applying heat treatment or storing them for a long period. Shogaols can change into paradols after hydrogenation.

Ginger contains a wide variety of other phenolic substances, including polyphenols, zingerone, quercetin, and gingerenone-A. This molecule is changed into 6-shogaol through dehydration and prolonged storage, which has more potent pharmacological effects and is more stable than its predecessor, 6-gingerol. Bacterial metabolism changes 6-shogaol into 6-paradol, and both have comparable anti-inflammatory properties.

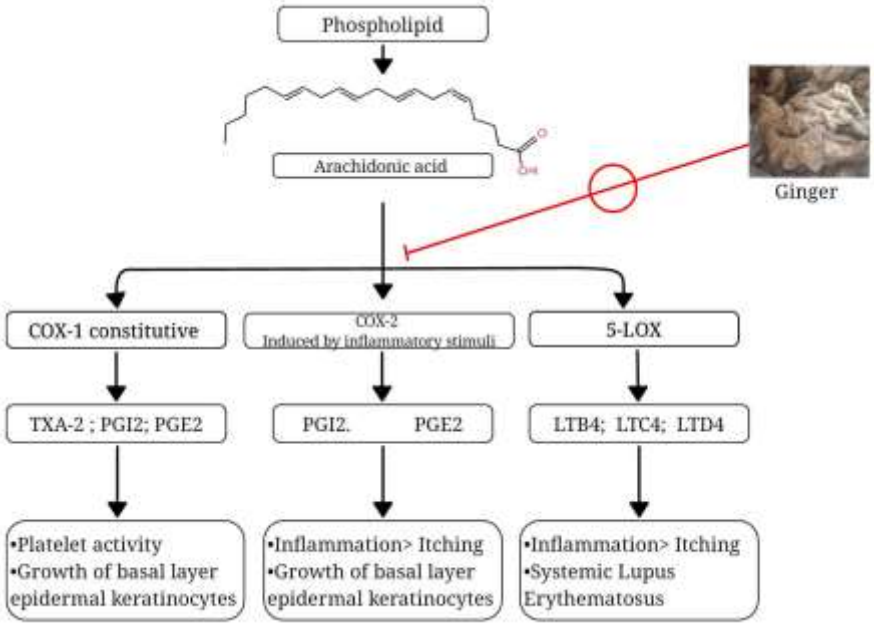

Figure 5: Anti-inflammatory(itching) mechanism action

Ginger suppresses inflammatory reactions via lowering NF-kβ, which in turn lowers the expression of cytokine genes. Ginger administered in liposomes for 21 days has been shown by multiple authors to lower TNF-α and IL-22 levels. (20)

In addition to demonstrating a significant decrease in Th2/1-mediated inflammatory cytokines, IgE, TNF-α, IFN-γ, thymus and activation-regulated chemokines, IL-1, 4, 12, and 13, cyclooxygenase-2, and nitric oxide synthase levels, 6-shogaol also inhibited the development of DNCB-induced Allergic Dermatitis-like skin lesions and scratching behavior. Via nuclear factor erythroid 2 related factor 2 (Nrf2) activation, 6-shogaol enhanced the levels of total glutathione, heme oxygenase-1, and quinone 1 while suppressing the production of reactive oxygen species and mitogen-activated protein kinases (MAPKs) signaling in vitro. 6-Shogaol inhibits immunological mediators via controlling the ROS/MAPKs/Nrf2 signaling pathway, which lessens Allergic Dermatitis-like lesions. (21)

4.2.7 Rosemary extract

Growing throughout the Mediterranean Sea and in the sub-Himalayan regions is the evergreen bushy shrub known as rosemary (*Rosmarinus officinalis L.*). The phytochemicals found in rosemary, including polyphenols, diterpenes, and monoterpenes. Ninety percent of the antioxidant potential of rosemary extracts is attributable to the conversion of carsonol from carsnic acid in the dry leaf. Other bioactive compounds with significant therapeutic potential that accompany carnosic acid include polyphenols (flavonoids, rosmarinic acid, and phenolic acids), triterpenes (ursolic acid and oleanolic acid), and diterpenes (rosmarol). The latter exhibit strong antioxidant properties. Rosemary exhibited higher UVA and UVB absorption power as well as higher anti-Staphylococcus aureus test activity. Because of the synergistic activity of its bioactive components to neutralize lipid peroxidation and reactive oxygen species (ROS), which cause cell damage, rosemary has strong antioxidant potential. The antibacterial activity and cytotoxicity of the compounds found in rosemary oil. (22)

4.2.8 Tea tree oil

Tea tree oil (TTO) is the volatile essential oil derived mainly from the Australian native plant *Melaleuca alternifolia*. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments. TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpinen-4-ol, γ-Terpinene, α-Terpinene,1,8-Cineole, Terpinolene, ρ-Cymene, α-Pinene, α-Terpineol, Aromadendrene, δ-Cadinene, Limonene, Sabinene, Globulol, Viridiflorol. (23)

Tea tree oil is the greatest oil for hair growth and thickness because it facilitates the scalp's efficient absorption of nutrients and awakens dormant hair follicles. tea tree oil was significantly superior to minoxidil alone and placebo in terms of stability, safety, and efficacy. (24)

4.3 Plant extraction methods

4.3.1 Extraction of Pomegranate peel

Organic solvents such as ethanol, methanol, ethyl acetate, chloroform, and ether can be used to analyse polyphenols chemically. Only ethanol is food grade among these, and it is mostly utilized in combination with water to recover phytochemicals from agricultural products sources (25)

The method used for extraction determines the plant extracts' quality and extraction efficiency. Due to their simplicity and ease of use, conventional methods are widely used; however, they have drawbacks, including higher extraction times and lower extraction efficiencies, which can have detrimental effects on biomolecules due to extended exposure This issue might be solved by the Ultrasonication Assisted Extraction method (UAE), which breaks down the cell wall and increases mass transfer rates by forming microcavities. This results in higher product yields with shorter extraction times and less solvent consumption. (26). Additionally, combining traditional and contemporary techniques improves extraction effectiveness and lessens the harm that organic solvents have to biomolecules and the environment. According to the HPLC profile, ellagic acid, the primary nucleus of ellagitannins, is a thermostable bioactive molecule. Neither temperature nor the UAE had a discernible impact on the hydrolysis of ellagitannins. The combination of ultrasonication and dynamic maceration aided extraction process yields the best results for a high recovery of phenolic components from pomegranate peel. Compared to the individual extraction methods of dynamic maceration-assisted extraction and ultrasonication, the combined method's responses were all much greater. It appears that these two approaches work best when combined.

We prepared 3 concentrations (C1, C2, C3) of Pomegranate peel powder extract (PPE) and made 3 different formulations in which the anti-bacterial activity on *Staph. Epidermidis* was assayed and the best formulation was selected. Pomegranate peel powder was mixed with a 60:40 (v/v) ethanol–water solvent at a ratio of 2:50 g/mL (C1), 4:50 g/ml (C2), 6:50 g/ml (C3). For the maximum yield of PPE, it was discovered that a sonication period of 70 minutes, a sonication temperature of 61.8°C, and a stirring speed of 1000 rpm for dynamic maceration were ideal. (27)

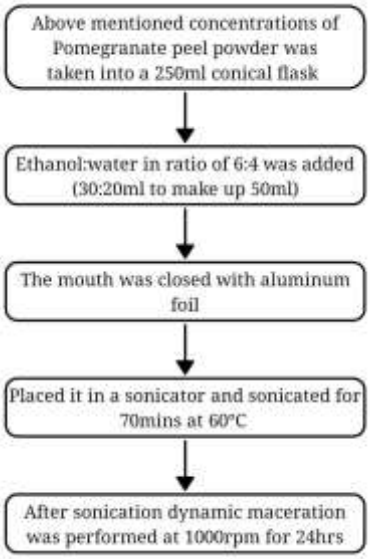

Figure 6: Pomegranate peel extraction procedure

4.3.2 Extraction of flax seed mucilage

The proportional yield of FG from flaxseeds can vary depending on which of the four procedures used to extract it—hot water extraction (9.0%), ultrasound-assisted extraction (7.8%), microwave-assisted extraction (7.0%), and alkaline-acidic extraction (6.4%). Hot water extraction yields the highest yield of FG, whereas the ultrasound-assisted extraction method is best suited for maximum purity. (12)

Xing et al. 2015 He looked into how the rheological characteristics of FG were affected by the extraction temperature. According to their findings, the gel's protein and polysaccharide content rose with temperature and peaked at 70 °C. Gum extracted at 70 °C and 80 °C revealed no discernible differences in viscosity. Given that 70 ◦C is insufficient to eradicate all germs and deactivate all gum-containing enzymes, a temperature of 98 ◦C may be chosen instead. Compared to 98 ◦C, the FG yield after extracting at 70 ◦C is lower. Regretfully, compared to the product recovered at the lower temperature, the product retrieved at a greater temperature is darker.

In our study we used 100ml of distilled water heated to 70 ◦C, then 10 g flaxseed was added. The mixture is maintained at 70 ◦C with stirring for 1 h, and FG was separated from the seed by filtering through a cotton cloth to obtain flax seed mucilage. (28)

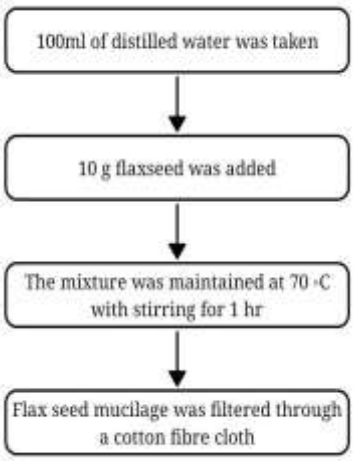

Figure 7: Flaxseed mucilage extraction procedure

4.3.3 Preparation of shampoo

- Take the required amount of flaxseed gel
- Add the above extracts in ascending order while stirring under propeller or with a magnetic stirrer.
- Finally measure the needed quantity and pack it in a bottle.

4.4 Formulations

Table 4: list of formulations made

INGREDIENT	FORMULATION 1 (F1)	FORMULATION 2 (F2)	FORMULATION 3 (F3)
Pomegranatepeel powder extract	5ml (C1)	5ml (C2)	5ml (C3)
Reetha extract	2ml	3ml	4ml
Shikakai extract	2ml	3ml	4ml
Rosemary extract	2ml	3ml	4ml
Tea tree oil	2ml	3ml	4ml
Ginger oil	2ml	3ml	4ml
Flax seed gel	q.s	q.s	q.s
Vetiver root extract	2ml	3ml	4ml

4.5 Physical evaluation

The formulations were evaluated in terms of their clarity and color.

4.5.1 Determination of PH:

The pH of 10% shampoo solution in distilled water was determined at room temperature 25°C using a pH meter after calibration. Most shampoos are neutral or slightly acidic. Acidic solutions cause the cuticle (outer layer) of the hair to shrink and lay flatter on the shaft of the hair. Basic solutions cause the cuticle to swell and open up. Acidic solutions make the hair seem smoother. Basic solutions make hair seem frizzier.

4.5.2 Determination of percentage solid content:

A clean dry evaporating dish was weighed and added 4 grams of shampoo to the evaporating dish. The dish and shampoo were weighed. The exact weight of the shampoo was calculated and the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated. If a shampoo has too many solids it will be hard to work into the hair or too hard to wash out. If it doesn't have enough, it will be too watery and wash away quickly. A good shampoo will have between 20% – 30% solids.

4.5.3 Surface tension measurement:

Measurements were carried out with a 10% shampoo dilution in distilled water at room temperature. Thoroughly clean the stalagmometer using chromic acid and purified water. Because surface tension is highly affected with grease or other lubricants. The data calculated by following equation given bellow:

$$R2 = (w3-w2)\ n1\ R1/\ (W2-w1)\ n2$$

Where, W1 is weight of empty beaker.
W2 is the weight of a beaker with distilled water.
W3 is Weight of beaker with shampoo solution.
n1 is no. of drops of distilled water.
n2 is no. of drops of shampoo solution.
R1 is surface tension of distilled water at room temperature.
R2 is surface tension of shampoo solution.

4.5.4 Cleaning action:

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

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

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

4.5.5 Wetting time:

The canvas was cut into 1 inch diameter discs having an average weight of 0.44 g. The disc floated on the surface of shampoo solution of 1% w/v and the stopwatch started. The time required for the disc to begin to sink was measured acutely and noted as the wetting time.

4.5.6 Foaming ability and foam stability:

Cylinder shake method was most widely used for determining foaming ability. 25 ml of the 1% shampoo solution was put into a 100 ml graduated cylinder and covered the cylinder with hand and shaken for 10 times. The total volumes of the foam contents after 1 minute shaking were recorded. The foam volume was calculated only. Immediately after shaking the volume of foam at 1-minute intervals for 4 minutes were recorded. (29)

4.6 Phytochemical screening

Preliminary phytochemical screening for the presence of tannins, alkaloid, glycoside, phenol, saponin and flavonoid were studied using the standard procedure as follows.

4.6.1 Tannins (Ferric chloride test):

To prepare the methanolic extract of plants, add a few drops of 5% aqueous ferric chloride solution. A bluish black colour indicates the presence of tannins.

4.6.2 Alkaloid (Hager's test):

Acidify 1ml of alcoholic extract of the plant with 1.5% of HCL and add few drops of Hager's reagent. A brown precipitate indicates positive test for alkaloids.

4.6.3 Glycoside (Benedict's test):

Dissolve a small amount of alcoholic extract of plants in 1ml of water and add 1 normal (N) sodium hydroxide (NaOH) solution. Yellow colour indicates the presence of glycoside.

4.6.4 Carbohydrates (Molisch's test):

The test solution is combined with a small amount of Molisch's reagent (α- naphthol dissolved in ethanol) in a test tube. After mixing, a small amount of concentrated sulfuric acid is slowly added down the sides of the slopping test tube, without mixing, to form a layer. A positive reaction is indicated by appearance of purple red ring at the interface between the acid and test layers.

4.6.5 Polyphenols (Sodium hydroxide test)

To 1ml of extract add few drops of dilute sodium hydroxide solution. Yellow colour indicates the presence of polyphenols.

4.6.6 Polyphenols (Lead acetate test)

To 1ml of extract add lead acetate solution. Yellow colour precipitate indicates the presence of phenols.

4.6.7 Sterols (Liberman butchard test)

To the extract add few drops of glacial acetic acid and 2 drops of sulphuric acid. Reddish brown colour indicates the presence of sterols. (30)

4.7 Determination of active compounds by UV Spectroscopy

Analytical studies of herbal drugs and formulations are essential to get quantitative and qualitative data for the presence of active constituents. To obtain the analytical data various instrumental techniques like spectroscopy, chromatography, electrophoresis, thermal methods, chemiluminescence and hyphenated techniques are widely used. We employed Ultra Violet Spectrophotometry for our analysis, it is a method used to measure absorbance of light by measuring the intensity of light passing through the chemical substance.

4.7.1 Sample preparation

The PPE obtained in 4.2.1 materials and methods by ultrasonication-dynamic maceration, it is set for Characterization under UV analysis.

4.7.2 Instrument and UV analysis

Tannins (Polyphenol) analysis was performed using a Lab India UV/VIS T60 spectrophotometer, Duo split beam, light source is tungsten lamp. (Lab India Instruments, model T-60, India) equipped with M. Wave professional software having the wave length acquisition range of 200–1200 nm. (31, 32)
Sample was diluted in ethanol and analysis at 200nm – 400 nm was done after blanking under following parameters: -
Photometric Mode: Absorbance
Response Mode: Normal
Range: 400.0 -- 200.0 nm
Interval: 1.0 nm

4.8 In-vitro assay evaluation

The bioactivity of the formulated hair shampoo, namely its antimicrobial activity was evaluated.

4.8.1 Media, antibiotics and bacteria

Disposable petri plates, glass rod spreader, Muller hinton agar, Staphylococcus epidermidis were kindly provided by MicroN life sciences. Clindamycin (300mg/ml) by Pfizer was purchased from a local pharmacy.

4.8.2 Procedure

- Anti-bacterial activity was performed using agar well method on *Staph. epidermidis.*
- Muller Hinton Agar plates were prepared.
- Two petriplates were taken as plate-1 and plate-2, *S. epidermidis* was spread over the plates and 5 wells were made using a borer with 400ul capacity.
- Varying concentrations of shampoo were made from F3 and were dispensed into the wells. The shampoo concentrations and the concentration of the extract in it are given below.
- The plates were incubated at 37℃ for 24 hours and the zone of inhibition was measured. (33)

Table 5: Agar plate-1

Plate-1		
Well number	Shampoo quantity (ul)	Extract concentration (mg/ml)
1	50	1.2
2	100	2.4
3	200	4.8
4	400	9.6
5	control	Distilled water

Table 6: Agar plate-2

Plate-2		
Well number	Shampoo quantity (ul)	Extract concentration (mg/ml)
1	30	0.6
2	50	1.2
3	100	2.4
4	200	4.8
5	Clindamycin	32 (ug/ml)

V. RESULTS

5.1 Physical evaluation

Table 7: Evaluation of formulation for physical appearance and pH

Sr.No	Formulation	Physical appearance	pH
1	F1	Slight yellow, translucent	5.8
2	F2	Bright yellow, translucent	5.9
3	F3	Dark yellow, translucent	6

Table 8: Evaluation of formulation for surface tension and %solid contents

Sr.No	Formulation	Surface tension	% solid content
1.	F1	33.71	19.70
2	F2	32.42	20.75
3	F3	31.66	22.85

Table 9: Evaluation of formulation for wetting time and cleansing

Sr.No	Formulation	Wetting time	% Detergency
1	F1	181	60
2	F2	182	60.8
3	F3	180	70

5.2 Phytochemical evaluation

Table 10: Phytochemical screening for Pomegranate peel extract

Name of the plant	Tannins	Glycoside	Phenol	Steriod	Alkaloids	Carbohydrates
Punica granatum	+	+	+	+	+	+

5.3 Determination of active compounds by UV spectroscopy

Many organic compounds give more than one maximum peak when its UV-Vis spectra is analysed. Each peak corresponds to an electron transition from a ground state to an excited state, and more than one different transition (with different energy, and therefore, different wavelength) are allowed.

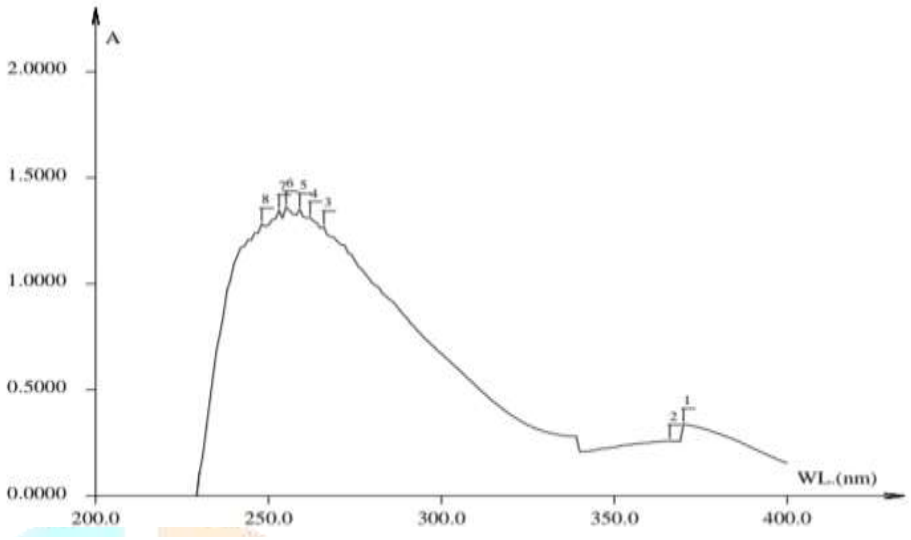

Figure 8: UV-VIS Analysis

Table 11: UV Characterisation of active compounds (31,32)

ID no.	Wavelength (nm)	Abs	T%	Compound
2	366	0.2601	54.95	Ellagic acid
3	266	1.2686	5.39	Galloyl-HHDP-hex
4	262	1.3126	4.87	Gallagyl-hex (punicalin)
5	259	1.3515	4.45	HHDP-gallagyl-hex (punicalagin)
6	255	1.3623	4.34	Ellagic acid-pent
7	253	1.3457	4.51	Ellagic acid
8	248	1.2822	5.22	Galloyl-HHDP-gluc (lagerstannin C)
9	224	-0.2930	196.34	……

5.4 Antibacterial evaluation by agar well diffusion method

Table 12: Result for agar plate-1

Plate -1	
Well number	Zone of Inhibition (cm)
1	1.5
2	2.2
3	2.5
4	2.9
5	-

Table 13: Result for agar plate-2

Plate -2	
Well number	Zone of Inhibition (cm)
1	1.2
2	1.7
3	2.0
4	2.5
5	2.3

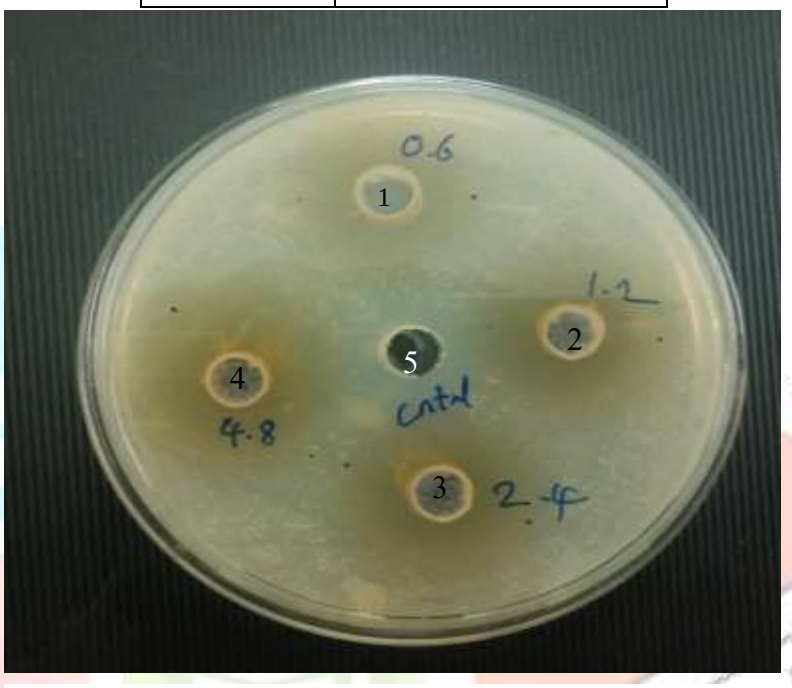

Figure 9: Zone of inhibition of plate 1

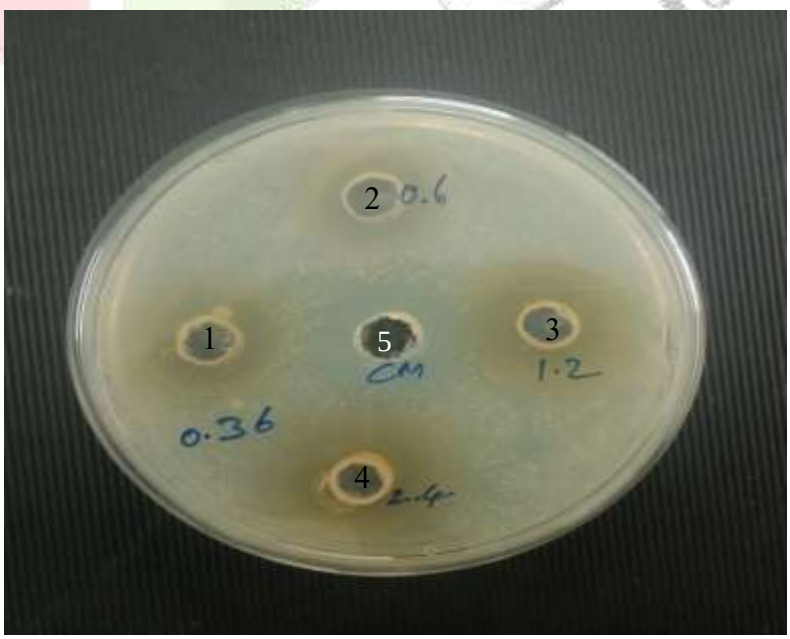

Figure 10: Zone of inhibition of plate 2

VI. DISCUSSION

The above literature was focused on formulation and evaluation of the anti-dandruff shampoo from pomegranate peel extract, no study for product stability or accelerated stability were done. It would be beneficial to conduct compatibility studies also in later studies. For future study it would be useful to analyse the stability and compatability issues which may help in probable use in its commercial manufacturing.

VII. CONCLUSION

The F-3 had lesser wetting time and had better detergency compared to F-1 and 2. The solid content was 22.85 which was higher than F1 and F2 but is within the specifications. It decreased the surface tension better than F1 and F2. The pH was under acceptable range according to the skin pH.

The UV analysis showed the presence of Ellagitannins and the main active tannin compounds Punicalagin and Punicalin which have been proven in various studies to have anti-bacterial action on *Staphylococcus epidermidis* which is a part of contributor for dandruff.

The Shampoo showed antibacterial response at all employed concentrations and 200ul of the shampoo was found to have better zone of inhibition than Clindamycin 32ug/ml.

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