



# INVITRO ANTIDANDRUFF ACTIVITY OF POLYHERBAL ECO-FRIENDLY HERB CARE COSMETICS WITH COMMERCIAL DANDRUFF SHAMPOOS

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## ABSTRACT

Dandruff is a random scalp disorder which is caused by the multiple different organisms of the family of Malassezia. The most efficient way to control dandruff is shampooing the scalp with the frequent intervals. Dandruff is a common scalp disease, that affects above 50% of the earth's population. The adults are the major affected people of seborrheic dermatitis. Dandruff is the major initial problem that induce the hair fall. The causative organism of dandruff is Malassezia furfur, it is a lipophilic, basidiomycetous fungi. As dandruff is seen in worldwide, it needs an effective treatment to overcome from the disorder. The numerous types of commercial antidandruff shampoos were used to treat dandruff as it contains some antifungal agents. As these shampoos has high concentrations of chemicals, they cause many side effects. Therefore, the polyherbal extracts prepared from different herbal plant materials were used to overcome from this problem. The antifungal activity of commercial antidandruff shampoos and the polyherbal extract shampoos were compared. The polyherbal hair care cosmetic was prepared by the different herbal plant materials, the extracts of soap pod, neem leaves, henna, hibiscus leaves, lemon peel, amla, curry leaves, green gram, Bengal gram, extract of rose petal, fenugreek seeds were dried and used as a natural polyherbal shampoo, which does not have any side effects. The antifungal assay was studied under the agar well diffusion and disc diffusion method. The phytochemical analysis of the polyherbal plant extract were carried out in this study.

**KEYWORDS:** Dandruff, Malassezia furfur, Polyherbal extract, Synthetic shampoo

## INTRODUCTION

A frequent recurrent inflammatory illness that affects the sebaceous gland-rich areas is dandruff. *Pityriasis capitis*, also known as dandruff, is a disorder marked by minute, white skin scale flakes that split and fall off the scalp [Rekha et al,2022]. Chronic scalp inflammation known as dandruff is characterised by scaling, itching, and redness of the scalp. It happens when the scalp sheds epidermal cells in bulk. A month or so, the scalp's skin goes through a renewal process. Normally, dead cells on the scalp are lost in an almost imperceptible manner, but occasionally, cell turnover is unusually quick, and dead cells are released as noticeable flakes that are referred to as dandruff (Loden&Wessman, 2000). A scalp condition known as dandruff is marked by an excessive loss of skin cells from the scalp. It is a widespread issue that affects people of all ages. Cells are propelled outward as the epidermal layer continuously replaces itself, until they finally perish and peel off.

These skin flakes are typically too minute to be seen by most people. Cell turnover can, however, be extraordinarily quick under certain circumstances, particularly in the scalp. According to a theory, skin cells may grow and shed for those with dandruff in 2–7 days as contrasted to those without it in about a month. Dead skin cells are thus released in big, greasy clumps, appearing as white or grey flakes on the scalp, skin, and clothing. The organism that causes dandruff is the yeast-like lipophilic basidiomycete fungus *Malassezia furfur*[*Pytirosporumovale*] [Obasichinelo et al,2018]. Previously known as *Pityrosporum*,*Malassezia species* is a lipophilic, dimorphic opportunistic yeast that causes dandruff, seborrheic dermatitis, and other skin and hair disorders. *Malassezia species* including *M. furfur*, *M.globosa*, and *M.restricta* are the culprits behind dandruff, also known medically as *Pityriasis capitis*. It is a frequent scalp condition that is also a significant cosmetic issue because it results in hair loss. There is currently no known complete treatment for this illness, according to investigations and reports [Naga padma et al, 2015].

The sebum lipid is transformed by *Malassezia* into fatty acids and triglycerides, which speed up the hyperproliferation of keratinocytes [Obasichinelo et al,2018]. Ayurveda has powerful natural treatments for controlling dandruff, but at the moment, people rely on commercial shampoos that incorporate antifungal ingredients like miconazole, ketoconazole, selenium sulphide, etc. Alkaloids, flavanoids, tannins, terpenoids, and other chemicals found in plant products have potent antifungal activity [Naga padma et al,2015]. To treat dandruff, many antifungal ingredients are used in hair care products. These medications have a number of negative side effects, including baldness, increased scaling, itching, irritation, nausea, and headache.

Consequently, an effort was made to create a synthetic anti-dandruff shampoo that is efficient in terms of safety and curing the dandruff problem [Sarovar Reddy et al, 2018].

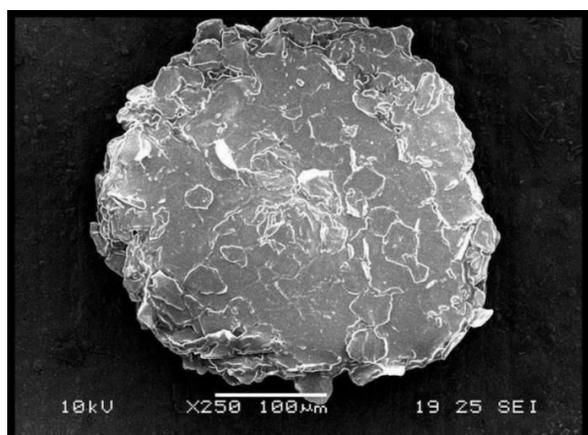
More than 50% of adults suffer from dandruff, which accounts for 25% of all scalp problems. Young adulthood is the starting point for dandruff, which lasts until middle age. It is frequently made worse by seasonal changes, trauma, variations in humidity, and emotional stress. More men than women are impacted. After age 50, rates start to decline [Rekha et al,2022]. In both wealthy and

underdeveloped nations, dandruff is a significant cosmetic issue that carries serious public health risks **(Krishnamoorthy et al., 2006)**. Dandruff is divided into two types: Dry (common) and Oily depending on the symptoms. *Pityriasis simplex*, another name for dry dandruff, is characterised by an excessive buildup of tiny scales with a white, grey, or ashen appearance on the scalp regions. These scales initially only affect the centre of the scalp before moving to the parietal, frontal, and temporal regions. There is no significant hair loss seen with this type of dandruff. There is no severe hair fall found in this kind of dandruff **[Mistry Zoya et al,2016]**. Numerous fungistatic substances have been demonstrated to reduce dandruff. Imidazole derivatives like ketoconazole and other substances like selenium sulphide, zinc pyrithione (ZnPTO), piroctoneolamine, ciproxirolamine, etc. are among the key active components. The main objectives of anti-dandruff products are to eliminate scales, lessen the adherence of *Malassezia (Pityrosporum) spp.* to corneocytes, and prevent yeast growth.

A wide variety of herbal substances, in addition to chemical ingredients, have been shown to have effective anti-pityrosporum or anti-dandruff (AD) activity, including pepper extract, basil extract, neem extract, rosemary oil, basil oil, clove oil, coleus oil, and tea tree oil **[Prabha Manju et al, 2009]**. The essential component of human charm is hair. Since ancient times, people have used a variety of herbs for controlling, washing, and adorning their hair. Synthetic substances have dominated throughout time, but people are now becoming more conscious of their negative impacts on skin, eyes, and hair **[Rakkimuthu et al, 2019]**. Shampoo is a cosmetic product that is used to cleanse the hair and scalp. It comes in an easy-to-use container. Its main purpose is to rid the hair of deposited sebum, scalp impurities, and hair-grooming product residue **(B.M.Mithal, 1994; ShobhaRani.R.Hiremath,2008)**. Around 80% of people worldwide, mostly in underdeveloped countries, utilise herbal medicines for primary healthcare, as reported by the World Health Organization. This approach of plant-based traditional medicine still plays a crucial part in healthcare **(V P Kapoor, 2005; Kunda B. Patkar, 2008)**.

For those who want to stay away from synthetic fungicides, there are herbal anti-dandruff shampoos available. These shampoos frequently contain botanical extracts or essential oils. Plant-based components and essential oils are found in ayurvedic anti-dandruff shampoos. Dust and dandruff are removed, the hair shaft is given gentleness and fineness, and hair fall is prevented. Several bacterial and fungal illnesses can be treated with floral extracts **[Rakkimuthu et al,2019]**. There is currently no known complete treatment for this condition, according to investigations and reports. Due to its widespread occurrence, this condition requires an efficient medicinal solution. Ayurveda has successful natural treatments for dandruff, but at the moment, people rely on commercial shampoos that contain antifungal ingredients **[Naga padma et al,2015]**. Plant extracts are known for their conditioning properties in addition to their anti-fungal properties, which will be helpful in maintaining the general health of the scalp and hair. According to earlier literature, dandruff is caused by the fungus *Malasseziaglobosa* (previously known as *Pityrosporumovale*) **[Obasichinelo et al,2018]**. Even now, despite recent advancements in science and technology, herbs are frequently employed as therapeutic agents. One of the nations in this manufactured globe that is abundant in a wide variety of medicinal plants is India. Due to their

accessibility, affordability, relative safety, and public trust, traditional (or herbal) medicines are currently encouraged, recommended, and promoted in national health care systems by WHO. One of the most crucial aspects of modern personal grooming is keeping the hair and scalp clean. Since soap-based shampoos were first introduced, a relatively new cultural phenomenon, the emphasis on cleanliness has grown, and the use of synthetic shampoos has also expanded. Anionic, cationic, and non-ionic surfactants are the major surfactants used in synthetic shampoos [Mohamed Halith et al,2009]. The market for conventional shampoo is heavily dominated by shampoos with a synthetic origin. Although the shampoo's primary purpose is to clean or act as a detergent, one of the crucial characteristics of an effective shampoo is its ability to remove dandruff. There is a need to develop formulations for preventing hair damages that comprise herbal extracts because people today's hectic lifestyles have made it difficult to guard hair and maintain hair homeostasis. Shampoos are used to keep hair maintained, making it softer, glossier, thicker, and longer, as well as to get rid of oiliness [Kothari Shreya et al,2018].



**IMAGE OF DANDRUFF FLAKE**

Infestations of bacteria and fungi on the scalp can result in conditions like *Tinea capitis* and *Pediculosis capitis*. Scalp problems come in a variety of forms, some of which are regular and others unusual. These consist of

Skin irritation on the scalp known as seborrheic dermatitis is characterised by scaly, scratchy, flaking skin.

Scalp psoriasis is a prevalent skin condition that affects the scalp and is characterised by raised, reddish patches that may also appear on the forehead, the back of the neck, or the ears.

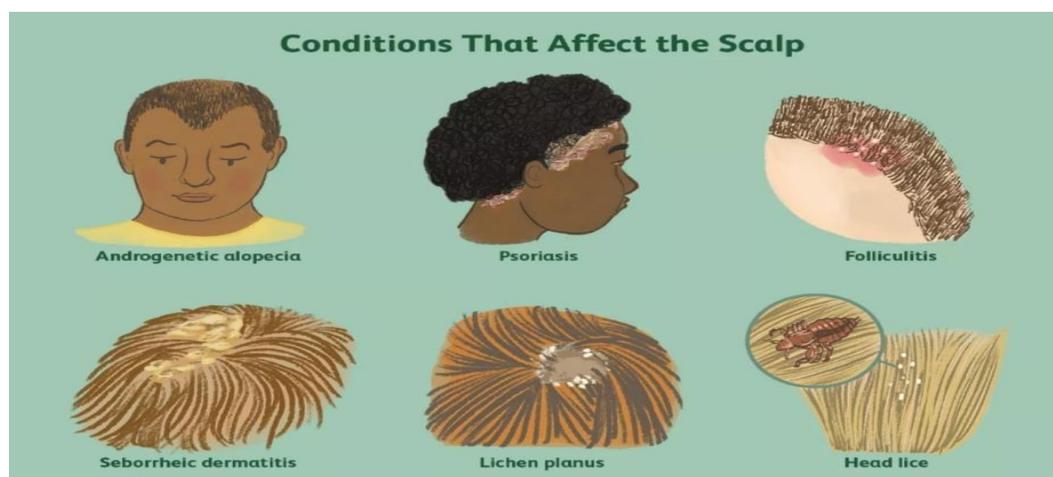
An infectious illness known medically as *Pediculosis capitis*, or head lice, is brought on by the obligatory parasite *Pediculus humanus capitis*, which lives on human scalps and feeds on human blood.

*Alopecia areata* – patchy baldness, loss of hair

The fungus *Trichophyton rubrum*, which causes ringworm, also goes by the name *Tinea capitis* and causes a cutaneous fungal infection of the scalp. Dead skin cells cause the discharge of dead skin cells, causing dandruff.

Hair follicle swelling is known as scalp folliculitis. Depending on each person's susceptibility, several factors determine the cause of dandruff.

There are two types of causes: microbial and non-microbial [Narshana et al,2018].



## ANALYSIS OF HAIR

The fatty layer of the scalp contains the hair follicles that generate hair. Unlike common perception, hair does not originate as a single strand but rather as groups of 1-4 hairs known as "follicular units." Thus every hair follicle has a hair bulb at the root in which the hair-growing process takes place. The dermal capillaries provide nutrients for hair follicles. The cells multiply and grow to create the hair shaft. The cells multiply and grow to create the hair shaft. Under the epidermis, the hair continues to grow while maintaining a delicate shape. The outer layer of hardens into keratin after it pushes through the epidermis [Sravanti et al,2021]. The epidermis has a component called hair. Hair is composed of distinct active hair follicles, tubular epithelial down growths into the dermis, subcutaneous fat, and mesenchymal-derived dermal papillae within the skin as opposed to thin, flexible tubes of dead, fully keratinized epithelial cells on the outside.

The flat, overlapping cells that make up the hair shaft cuticle protect the hair from the epidermis' tip to its base. The cuticle cell typically has an apparent length of 50  $\mu\text{m}$  and a thickness of between 0.3 and 0.5  $\mu\text{m}$ .

The area of the follicle that actively generates hair is known as the hair bulb. It contains a single capillary loop, nerve fibres, the follicular dermal papilla, dermal papilla cells, mucopolysaccharide-rich stroma, and the dermal papilla. The hair bulb can be separated into two sections: the lower portion contains undifferentiated cells, and the top portion contains differentiated cells. The infundibulum and the isthmus are the two anatomical components of the upper hair follicle, which is located just above bulb [Barbara buffoli et al,2013]. Defects of hair growth are typically caused by decreased and/or aberrant creation, decreased regeneration, or larger destruction of Hair follicle, which has the effect of changing either the quality or the overall density of hair follicles (Stenn and Paus 1999; Al-Nuaimi et al. 2010). A fascinating area of study in the field of hair is the innervation and vascularization of hair. Although it was more challenging to describe the intricate architecture of nerves, both have been discussed in literature.

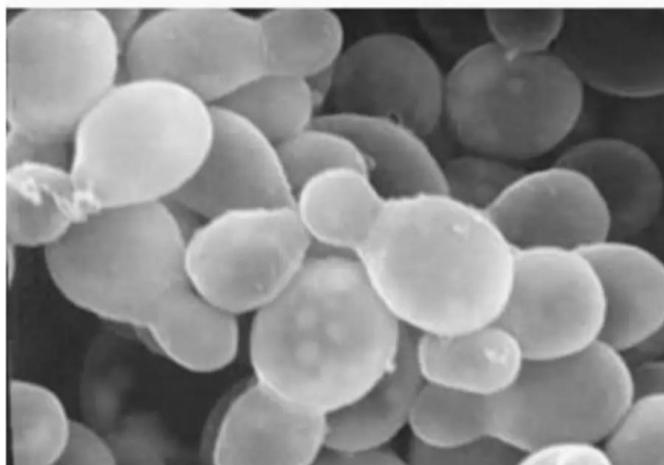
Hair-related issues include dandruff, Frayed ends, Unclean hair, Unruly hair, Limpid hair, Hair fall, Heat injury, Colour fading, A grey colour change of hair. Dry skin irritated oily skin, and not enough shampooing are the main causes of dandruff. Fungus that resembles *Malassezia* and sensitivity to hair products (contact dermatitis) [Sravanti et al,2021].

#### **ROLE OF MALASSEZIA SPECIES IN DANDRUFF**

Dandruff predominance in the general public ranges from 30 to 95 percent. In addition to the inconvenience, this ailment is unpleasant in social situations and lowers the individuals' esteem. *Malassezia* species, stress, exhaustion, harsh weather, greasy skin, shampoo use, immunocompromised condition (AIDS), and neurodegenerative disorders all contribute to the aetiology of this syndrome [Barbara buffoli et al,2013]. Dandruff is commonly thought to be caused by *M. restricta* and *M. globose*. Depending on where the host is located geographically, different animals are concerned. The other species linked to this syndrome include *M. furfur*, *M. sympodialis*, *M. obtuse* and *M. slooffiae*. By expressing lipase, *Malassezia furfur* breaks down the triglycerides in sebum, producing oleic acid as a lipid byproduct. A polyherbal mixture that might be added to shampoos or hair oils for increased anti-dandruff effectiveness.

Fungal infections known as superficial mycoses attack the keratinized layer of human skin and hair follicles. Most of the time, these infections are not symptomatic and lack cellular and/or humoral responses. A number of species of *Malassezia*, a normal flora, cause the chronic and moderate superficial mycosis known as *Pityriasis versicolor*. *Pityriasis versicolor*, folliculitis, seborrheic dermatitis (SD), and atopic dermatitis are all linked to *Malassezia* species. *Malassezia* also colonise the healthy skin of several animals and humans. Seborrheic dermatitis (SD) is a common skin condition that is characterised by the appearance of erythematous patches [Ali Zarei-Mahmoudabadi et al,2013].

## *Malassezia furfur*



### FORMULATION OF SHAMPOO

Although it was always believed that an excellent shampoo not only leaves hair clean, rinses out dirt from it, and gives it gloss, but also makes hair controllable and non-dry. However, at the moment, shampoos' ingredients and intended uses are so diverse that no single classification can encompass everything altogether [Sarovar Reddy et al,2015]. The most popular method of treating hair and scalp issues is shampoo. The sole accessible cleaner for hair up until the first non-alkaline shampoos were released in 1933. One expects modern shampoos to be considerably more than just cleaning agents. They should produce lather in both hard and gentle water and yet when treated to oily hair, without drying out the hair[Ralph M. Trueb,2006]. According to Khaloud Al Badietal, shampoo is typically a detergent solution with acceptable additions for additional benefits like improved hair conditioner, lubricating, medication, etc[Khaloud Al Badi et al,2014]. There are numerous synthetics, herbal, medicated, and non-medicated shampoos on the market today.

Presently, using shampoo to wash one's hair and scalp is a fairly ubiquitous practise. Its main purpose is to rid the hair of deposited sebum, scalp impurities, and hair-grooming product residue. The final need is that the entire shampoo formulation be long-term, medically safe [Pooja Arora et al,2011]. Synthetic surfactants are usually added to shampoo for the foaming and washing effects, but continuous use causes hair to become dry, hair to fall out, and the scalp and eyes to get irritated (Potluri et al., 2013) Several quality management procedures, such as visual evaluation, physicochemical controls, and conditioning performance analysis, were carried out to assess the grade of commercial and created formulations [Ashok and Rakesh, 2010]. Scalp scaliness caused by dandruff is a non-inflammatory disorder, but seborrheic dermatitis is an inflammatory condition that causes redness, itching, and scaling. *Malassezia fungus*, sebum lipids, and individual sensitivity are the three most common etiologic factors that have been identified for both of them.

### SEBORRHEIC DERMATITIS



## CLASSIFICATION OF SHAMPOOS

- Powder shampoo
- Liquid shampoo
- Aerosol shampoo
- Jelly shampoo
- Cream shampoo
- Keratin shampoo
- Specialised shampoo
- Volumizing shampoo

### POWDER SHAMPOO:

It is supplied as dry powder; formerly, dry soaps were used to make it, but today dry synthetic detergents are utilised in its creation. When making powder shampoo, the activity of the ingredients is reduced by the addition of water or another solvent, especially when making medicated shampoo. These shampoos are no longer utilised in modern times because of the difficulties associated with using them.

### LIQUID SHAMPOO:

The most popular formulations are these transparent aqueous ones. Typically, low cloud point detergent is used to make them. These shampoos could include transparent varieties.

### AEROSOL SHAMPOO:

As they are packaged in aerosol jars, these shampoos are known as aerosol products. They require special composition, processing, and packaging because an additional propellant is used. The propellant that is added needs to work well with the cleansing components and shouldn't inhibit their activity. An aperture for the container has a valve. When the valve is depressed, foamy shampoo is released. Consequently, aerosol shampoo is also known as foam type shampoo.

**JELLY SHAMPOO:**

These are often created using a gelling agent and are translucent and dense (e.g., cellulose). Detergent, which can be utilized either alone as well in conjunction with soap, is the main component. The detergent concentration can be changed to produce gel with the desired consistency. Gel shampoo can also be created by thickening clear liquid shampoo after adding methyl cellulose to it. Hair salons and beauty salons make extensive use of it.

**CREAM SHAMPOO:**

These products are a variant of clear liquid cream shampoos and are referred to as lotion shampoos. The additional opacifier is also dissolved using solubilizing agents like magnesium stearate.

**KERATIN SHAMPOO:**

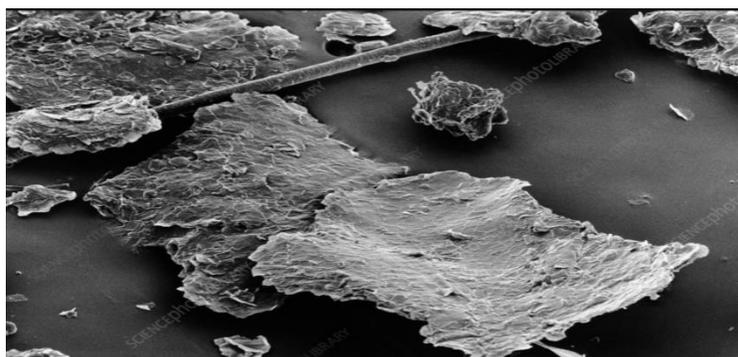
The advantages that come from using shampoo (or any other hair care product) enriched with keratin oil moisturize and condition the hair. This makes it appear silky and polished. Additionally, it aids in reducing frizz, controlling fly aways, and guarding against harm from styling appliances like such a flattening iron or blow dryer.

**SPECIALISED SHAMPOO:**

Persons having dandruff, colour-treated hair, gluten or wheat allergies, a desire to use a natural ingredients, newborns, and youngsters are among the target markets for specialty shampoos "baby shampoo" is less irritating

**VOLUMIZING SHAMPOO:**

Volumizing shampoos give hair a fuller, bouncier, and more full-bodied appearance. It has more to do with the hair's texture than its strand thickness. Instead, volumizing shampoos ought to be light enough not to push down your hair, which will ultimately give it more body [Sravanti et al,2021].

**DANDRUFF FLAKES**

The skin contains specific ecological niches in each area. The dense terminating hair, numerous sweat and sebaceous glands, and a high humidity level of the scalp make it a highly favorable environment for microbial colonisation. A nutrient-rich environment is created by the SC maturing, which produces a sufficient quantity of amino acids, sweat glands secreting minerals, and sebaceous glands producing sebum.

## BASIC INGREDIENTS OF SHAMPOO



## EVALUATION OF SHAMPOOS

The formulation has undergone the following physical assessment. They are

- Detergents
- pH
- Foaming agents
- Viscosity
- Stability
- Sequestering agent
- conditioners
- Additives
- Thickeners and opacifiers

**DETERGENTS:**

These surfactants mix, emulsified, and wash the filth and grease away (soil). Different kinds of surfactants, such as sodium olefin sulphate, triethanolamine lauryl sulphate, and laurel/laureth sulphates, are designed to clean different kinds of hair. Additionally, detergent would strip the hair of sebum in order to thoroughly clean them, leaving them dry.

**DETERMINING P<sup>H</sup>:**

They must work well with the surfactants. Alkanol amides were the first agents utilised, although different aqueous and non-aqueous agents have since taken their place. They must work well with the surfactants. Alkanol amides were the first agents utilised, although different aqueous and non-aqueous agents have since taken their place. They must work well with the surfactants. Alkanol amides were the first agents utilised, although different aqueous and non-aqueous agents have since taken their place. A 10% volume by volume shampoo solution in distilled water was measured using a PH metre under the following conditions: room temperature [Shreya Kothari et al,2018].

**P<sup>H</sup> ADJUSTERS:**

With alkaline detergents, damaged hair might swell, so an acidic pH adjustment is made when creating shampoos that are appropriate for such hair. Citric and lactic acids are among the ingredients.

**FOAMING AGENTS:**

These are widely used in shampoos because traditionally, people have associated potency with shampoo foaming. Shampoo must be used several times on greasy hair before it properly foams. Column height is said to be proportional to volume.

**STABILITY:**

Samples maintained at ambient temperature (25°C and 45°C) and in the refrigerator (4°C) as well as samples packaged in glass vs plastic containers were examined for the antifungal activity of the formulations.

**SEQUESTERING AGENTS:**

When used with soft water, a detergent leaves a gummy film on the surface. The hair becomes lifeless and unmanageable because to this muck. When soap is used to wash the hair, this is frequently observed. Sequestering chemicals chelate the calcium and magnesium ions in hard water to stop the growth of this scum.

**CONDITIONERS:**

A powerful cleaner will completely remove all grease from the hair, leaving it dry and dull. Therefore, shampoos include a variety of cuticle-coating chemicals to enhance shine, slide, and easy combinability.

**ADDITIVES:**

Depending on the type of detergent used, various extra agents are added to shampoos to either improve conditioning or to distinguish them as natural or herbal shampoos. Shampoos are also marketed for different hair types. When shampooing, the hair is thoroughly wetted, a generous amount of shampoo is placed in the palm, and the scalp and thickness of the hair are then covered. Before using shampoo, hair shouldn't be gathered on the back of the head as this can cause matting.

**THICKENERS AND OPACIFIERS:**

These are aesthetically pleasing to the user however they do not enhance the purifying effect. They must work well with the surfactants. Alkanol amides were the first agents utilised, although different aqueous and non-aqueous agents have since taken their place.

**CAUSES OF DANDUFF**

The fungus *Pityrosporumovale*, which again is naturally found on the scalp and other areas of the skin, may be the cause of dandruff. Usually, this fungus doesn't harm anything. However, as a result of hormonal changes, stress, and changes in the weather, the scalp will create more oil, which will lead to the growth of the fungus *P. ovale*. The spread of the fungus causes itchy scalp skin cells, hair follicle loss, and what is often known as "dandruff." There will be a loss of hair follicles and apparent dandruff. It is currently thought that the synthesis of lipase enzymes is the precise process by which dandruff develops.

**SYNTHETIC ANTI DANDRUFF SHAMPOO**

These formulations contain anti-dandruff drugs for therapeutic use, which are divided into three classes based on their mechanisms of action. Fungicidal chemicals, such as imidazoles and zinc pyrithione, Cytostatic agents include tar, selenium sulphide, and octopirox. keratolytic chemicals, such as sulphur compounds and salicylic acid.

**MERITS OF HERBAL SHAMPOOS**

- It uses organic ingredients and is pure.
- without side effects.
- No synthetic ingredients are used.
- No substances derived from petroleum are used.
- It is kind to skin.

## DEMERITS OF SYNTHETIC SHAMPOOS

- Sensitive individuals may experience significant irritation from the artificial ingredients used in synthetic shampoos.
- It causes cancer and even nervous system harm.
- The active components in synthetic shampoos can give you a headache, make you feel nauseous or lightheaded, or even make you throw up occasionally.
- It has greater negative impacts.

The sebum lipid is transformed by *Malassezia* into fatty acid triglycerides, which speed up the hyper proliferation of keratinocytes. Ayurveda has powerful natural treatment for controlling dandruff.

Hence to overcome the problem, the present investigation to evaluate the invitro antidandruff activity of polyherbal eco-friendly herb care cosmetics with commercial dandruff shampoos.

## MATERIALS AND METHODOLOGY

### COLLECTION OF PLANT MATERIALS:

All the plant materials were obtained from a local ayurvedic store. Collected plant parts were washed with tap water and dried in shade. Dried plant parts were powdered and stored in air tight container for further studies.

### COLLECTION OF SAMPLES:

The organisms were isolated from the person's scalp who suffering from dandruff flakes or scales, collected with a sterile comb and scrapping the hair. The specimens were transferred into a dark sampling paper to prevent exposure to sunlight.

### COLLECTION OF SHAMPOOS:

The commercial antidandruff shampoos were collected as sample from various standardized pharmacy and reputed super markets of Tiruvannamalai District. The batch numbers and expiry dates were noted with the presence or absence of the manufacture seals.

### LIST OF THE SHAMPOOS:

- Antidandruff Ketoconazole Shampoo
- Himalaya Antidandruff Shampoo
- Meera Antidandruff Shampoo
- Head and Shoulder Antidandruff Shampoo
- Dove Antidandruff Shampoo

**ISOLATION OF MICROORGANISMS:**

The SDA plate were prepared. The samples were inoculated over the surface of SDA with olive oil which was added with the antibiotic chloramphenicol to avoid bacterial contaminants. The plates were incubated at 28C for 7 days and regular observations were done.

**COLLECTION OF POLYHERBAL MATERIALS:**

S. No	Name of the Plant	Parts used	Scientific Name	Purpose
1.	Soap pod (Shikakai)	Dried fruit	<i>Acacia concinna</i>	Hair growth
2.	Neem	Leaves	<i>Azadirachta indica</i>	Hair Cleanser
3.	Henna	Leaves	<i>Lawsonia inermis</i>	Natural conditioner
4.	Hibiscus	Leaves	<i>Hibiscus rosa-sinensis</i>	Hair growth promoter
5.	Lemon	Fruit skin peel	<i>Citrus limon</i>	Preservative
6.	Amla	Fruit	<i>Phyllanthus emblica</i>	Antidandruff
7.	Curry leaves	Leaves	<i>Murrayakoenigii</i>	Antioxidant
8.	Green gram	Seeds	<i>Vigna radiata</i>	Antidandruff
9.	Bengal gram	Seeds	<i>Cicer arietinum</i>	Hair growth
10.	Rose petal	Flowers	<i>Rosa</i>	Natural cleanser
11.	Fenugreek	Seeds	<i>Trigonella foenum-graceum</i>	Antidandruff

**DIRECT MICROSCOPY TO LACTOPHENOL COTTON BLUE:**

- A drop of lactophenol cotton blue was introduced at the centre of a clean slide.
- Fragment of the fungus colony 2-3 mm from the colony using wire loop and the fragment was placed on the stain and teased gently.
- A coverslip was applied over the prepared glass slide.
- The preparation was observed under the low and high magnification.

**DIRECT MICROSCOPY:**

- A drop of 10% KOH was added onto a clean slide containing the smear of sample and the smear was covered with a cover slip.
- The slides were observed under 40X objective lens.

**PHYSICAL APPEARANCE AND CONTENT ANALYSIS:**

For the evolution of poly herbal eco-friendly hair care shampoo, it was assessed using various quality control tests such as  $p^H$ , physical appearance and determination of solid contents.

**PHYTOCHEMICAL ANALYSIS OF POLYHERBAL ECO-FRIENDLY HAIR CARE SHAMPOO EXTRACT:****TEST FOR GLYCOSIDES:**

- The 1ml of plant extract and 2ml of acetic acid, finally 1ml of concentrated  $H_2SO_4$  (Sulphuric acid).
- Tetra-oxy-sulphate is added drop by drop in the test tube.
- Formation of oil layer on top of the solution.

POSITIVE RESULT – Formation of oil layer

NEGATIVE RESULT – No formation of oil layer

**TEST FOR ALKALOIDES:**

- 3ml of plant extract added with 1ml of 1% HCL in the test tube.
- Few drops of Meyer's reagent were added in the test tube.

POSITIVE RESULT – Creamy white precipitate.

NEGATIVE RESULT – No creamy white precipitate formation appears.

**TEST FOR TANNIS:**

- The 1ml of plant extract and 2 drops of 5%  $FeCl_3$  was added in the test tube
- Formation of dirty green precipitate is the change that takes places in the test tube.

POSITIVE RESULT – Formation of dirty green precipitate.

NEGATIVE RESULT – No formation of dirty green precipitate.

**TEST FOR FLAVONOIDES:**

- The 1ml of plant extract and 3 drops of Ammonia ( $NH_3$ ) was added in the test tube.
- Add 0.5ml concentrated HCL in the test tube.
- Formation of pale brown colour is the change that takes places in the test tube.

POSITIVE RESULT – Formation of pale brown colour.

NEGATIVE RESULT – No formation of pale brown colour.

**TEST FOR STEROIDS:**

- The 1ml of plant extract and 1ml of H<sub>2</sub>SO was added in the test tube.
- Formation of red colour is the change that takes places in the test tube.

POSITIVE RESULT – Formation of red colour.

NEGATIVE RESULT – No formation of red colour.

**TEST FOR RESIN:**

- The addition of 5ml of plant extract with 5ml of copper acetate in the test tube.
- Shake vigorously, allowed to separate appearance of reddish brown precipitate in the test tube.

POSITIVE RESULT – Appearance of reddish brown precipitate in the test tube.

NEGATIVE RESULT – No appearance of reddish brown precipitate takes place.

**TEST FOR SAPONIN:**

- Add 2ml of plant extract with 5 drops of olive oil in the test tube.
- Formation of stable emulsion in the test tube.

POSITIVE RESULT – Formation of stable emulsion.

NEGATIVE RESULT – No formation of stable emulsion.

**BIOCHEMICAL TESTS:****INDOLE TEST:**

- Tryptophan broth was prepared, sterilized and dispensed into sterile test tubes.
- The test organisms were inoculated in the tubes of tryptophan medium.
- Incubate the test tubes at 37°C for 24 hours.
- After incubation of the test tubes, add 0.2ml of Kovac's reagent and gently shake the test tubes.
- Allow the tubes for minutes and observe the results.

Positive result – Formation of red ring.

Negative result – No formation of red ring.

**METHY RED TEST:**

- MR-VP broth was prepared, sterilized and dispensed into sterile test tubes.
- Inoculate the test organisms in the test tubes.
- Incubate the test tubes at 37°C for 24 hours.
- After the period of incubation, add 5-6 drops of methyl red solution and gently shake it.
- Allow the tubes for few minutes and observe the results.

Positive result – Formation of red colour.

Negative result – Formation of yellow colour.

**VOGES PROSKAVER'S TEST:**

- MR-VP broth was prepared, sterilized and dispensed into sterile test tubes.
- Inoculate the test organisms in the test tubes.
- Incubate the test tubes at 37C for 24 hours.
- After incubation, add 0.2ml of VP reagent A and 0.2ml of VP reagent B and shake the tubes.
- Allow the tubes for few minutes and observe the results.

Positive results – Appearance of red colour.

Negative results – Appearance of yellow colour.

**CITRATE UTILISATION TEST:**

- Simmon citrate agar was prepared, sterilized and dispensed into sterile test tubes.
- Slants were made with the test tubes and the test organisms were inoculated into the slants.
- Incubate the test tubes at 37C for 24 hours.
- After the incubation period, read results.

Positive result – Formation of Prussian blue colour.

Negative result – Formation of green colour.

**OXIDASE TEST:**

- Take a sterile oxidase disc, add a drop of test sample culture.
- By using the sterile applicator stick or glass rod, gently mix it.
- Observe the changes that takes places in the disc and read the results.

Positive result – Formation of purple colour.

Negative result – No colour changes take place.

**TRIPLE SUGAR AGAR TEST:**

- The Triple sugar iron agar medium was prepared, sterilized and dispensed into sterile test tubes.
- The agar slants were made and the test organisms are inoculated into the slants.
- Incubate the test tubes at 37C for 24 hours.
- After the period of incubation, observe the results.

**UREASE TEST:**

- The Christensen's urea agar medium was prepared, sterilized and dispensed into sterile test tubes.
- The agar slants were made and the test organisms are inoculated into the slants.
- Incubate the test tubes at 37C for 24 hours.
- After the period of incubation, observe the results.

Negative results – Formation of red colour.

Positive results – Formation of pink colour.

**CATALASE TEST:**

*Malassezia species* were detected using the catalase test because all of them, with the exception of *M. restricta*, are catalase positive. In a test tube, 3ml of a 3% hydrogen peroxide solution was added. A sterile glass rod was used to insert a number of the isolated fungal colonies into the test tube. The formation of the gas bubbles is caused by the catalase enzyme breaking down hydrogen peroxide into oxygen and water.

**ESCULIN HYDROLYSIS TEST:**

The medium used was bile esculin agar slant which is a nutrient agar, based medium containing 0.1% esculin and 10% A pure culture's inoculums were streaked along the slant and aseptically transferred into a sterile tube of bile esculin agar. The infected tube was kept in an incubator for 24 hours at 30C to ascertain the outcome. bile salts, and allowed to solidify as a slant. The bile salt inhibits some bacteria, and also shows the ability to grow in the presence of bile salts represents a second test use for the medium

**PREPARATION OF POLYHERBAL EXTRACT:**

All the ingredients collected, dried and powdered for preparing the extract. In 500ml beaker, add 10g of dried extract powder of herbs in 350ml of water and boil for 45 minutes. After, 45 minutes of heating followed by concentration, cool the extract for few minutes, filter using filter paper and collect the filtrate in conical flask and tightly cover with cotton plug.

**VISUAL APPEARANCE:**

The formulation underwent a visual inspection to assess their outside appearance. The created formulations were assessed for their viscosity, transparency, colour, and ability to produce foam.

**DETERMINATION OF P<sup>H</sup>:**

At room temperature, the P<sup>H</sup> of a 10% w/v shampoo solution in distilled water was measured. A P<sup>H</sup> metre was used to measure the P<sup>H</sup>.

**SURFACE TENSION MEASUREMENT:**

The surface tension of shampoos that had been diluted (10% w/v) in distilled water was measured at 20°C using a tensiometer, and the results were estimated using the equation below.

$$R2 = (W3 - W1)n1/(W2 - W1)n2 \times R1$$

- Where W1 is the weight of the empty beaker.
- W2 is the weight of beaker with the shampoo solution.
- N1 refers to the number of drops of distilled water.
- N2 denotes the number of drops of shampoo solution.
- R1 is the surface tension of distilled water at room temperature.
- R2 is the surface tension of the shampoo solution.

**WETTING TIME:**

The filter paper was divided into discs with a 1-inch diameter and a weight of 0.44 g on average. The disc floated on top of the 1% w/v polyherbal extract solution.

**MEASUREMENT OF VISCOSITY:**

The viscosity of the polyherbal extract was assessed using the Brookfield Viscometer LVDV Prime-I. At room temperature or 30±2°C, by using various rpm and torque, the viscosity of a polyherbal extract was tested.

**SURFACE TENSION MEASUREMENT:**

Adjust the 10% concentration by diluting the polyherbal extract with distillate water. With the use of a stalagmometer, measurements were taken. The generated polyherbal extract sample should be poured into a beaker and allowed to gently run from the mark using the flattened end of a stalagmometer. Fix it on the stand and give the sample time to progressively bleed away from the mark. When the liquid level moves from A to B, count how many drops are produced. With distilled water, repeat the experiment. The equation below was used to calculate the data.

$$R2=(W3-W1)$$

- Where, W1 is the weight of the empty beaker.
- W2 is the weight of beaker with the distilled water.
- W3 is the weight of the beaker with shampoo solution.
- N1 refers to the number of drops of distilled water.

- N2 refers to the number of drops of shampoo solution.
- R1 is the surface tension of distilled water at room temperature.
- R2 is the surface tension of shampoo solution.

### **PHYTOCHEMISTRY CLEANING ACTION:**

Grease was added to five grams of wool yarn, which was then put in a flask with grams of shampoo and 200 ml of water. The flask's content was kept at a constant 302°C temperature. At a pace of 50 shakes per minute, the flask was shaken for 4 minutes. The sample was taken out, dried, and weighed after the solution was removed. The following equation was used to determine how much grease was eliminated.

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

Therefore,

- DP refers to the percentage of detergency power,
- C denotes the weight of sebum in the control sample,
- T is the weight of sebum in the test sample.

### **STERILITY CHECK:**

- The silver nanoparticle of polyherbal eco-friendly hair care shampoo extract was streaked separately on Muller hinton agar and Nutrient agar to check the purity.
- After 24 hours of incubation the plates were used for bioassay.

### **PROCEDURE:**

- Nutrient agar and Muller hinton agar were prepared and poured in petriplates and allowed to get solidify.
- The silver nanoparticle of polyherbal eco-friendly hair care shampoo extract was streaked in the agar plates and incubate at 37 C for 24 hours.
- After incubation the plates were observed for any contamination.
- The contaminated silver nanoparticle of polyherbal eco-friendly hair care shampoo were discarded.

### **DILUTION OF COMMERCIAL SHAMPOO:**

The commercially available shampoos were diluted with sterile distilled water to get 10 folds, 20 folds and 30 folds

dilution. These were used for the antidandruff activities.

### **INVITRO ANTIDANDRUFF ACTIVITY OF POLYHERBAL SHAMPOO:**

Cup plate method was used for determining the antidandruff activity of shampoo. It was carried out by employing culture of fungi *Candida albicans* in sabouraud dextrose agar medium. The medium was poured into petri plate which was sterilized in autoclave. At room temperature, petri plate is allowed to solidify. The organism

was inoculated using loop and was spreaded using glass spreader. In each petri plate 3 wells were made using sterile cork borer. One was treated with standard (fluconazole) and other 2 wells with polyherbal shampoo. In the same way other 2 plates were prepared. The plates were incubated at 20-25°C. After incubation, plates were observed for zone of inhibition. The diameter of zone of inhibition was measured using ruler and compared with standard and recorded in mm.

### **ANTIFUNGAL ASSAY:**

The antidandruff activity of commercial antidandruff shampoo and polyherbal extract was tested by disc diffusion method and agar well diffusion method.

## **RESULTS AND DISCUSSION**

### **RESULTS**

A common ailment known as dandruff causes the scalp's skin to flake, neither dangerous nor contagious. Yet it can be awkward and challenging to treat. A mild daily shampoo can be used to treat mild dandruff. The oil content of the hair is removed by the washing action of synthetic shampoos, rendering the hair dry and wavy. Herbal shampoos, on the other hand, work to give the hair the essential nutrients it needs while also gently washing the hair to remove any extra oil.

The present study was carried out in Kamban College of arts and science for women, Thiruvannamalai. The collected samples were used for the isolation of *Malassezia* fungus.

The polyherbal extracts prepared from different herbal plant materials, which contains the essential antidandruff, antimicrobial, antifungal agents that not only cleanses the scalp and provides essential nutrients to our body.

The majority of the chemicals used to make fragrances pose a risk because they can cause cancer, infertility, rashes, itchiness, hair loss, and asthma. When present in your shampoo and conditioner, synthetic colours provide an additional risk. Petroleum and coal tar are the sources of colour. The most commonly used component in the synthetic shampoo, Sulfates: They cause dryness by making the skin more sensitive and by robbing the hair of its natural oils. The risk of skin cancer is raised by parabens. Phthalates cause hormonal changes and are hazardous to the environment. Formaldehyde: causes cancer and is readily absorbed through the skin.

Herbal shampoos for dandruff and hair growth are designed to nourish the roots and follicles of the hair while also strengthening the hair follicles. In turn, this encourages the growth of fresh, healthy hair roots and healthy hair growth. The culture was prepared by inoculating the flakes of dandruff in SDA broth, which was shown in the FIGURE 1. The colony morphology of the fungus was studied under the plate method by the Nutrient agar, Sabouraud dextrose agar. The organism grew as white to tan cream coloured colony with smooth

pasty consistency on SDA. The results were shown in FIGURE 2. The cells appeared bottle-shaped in the microscopical observation. The results were shown in FIGURE 3. The organism was further screened by LPCB, Direct microscopy and biochemical test.

The isolation and identification of the fungi *Malassezia furfur*, was carried out by the Lactophenol cotton blue staining and KOH wet mount. The organism shown as meat ball shape on LPCB staining and KOH mount.

The identification of the *Malassezia* was carried by biochemical test. The dextrose and xylose fermented and produce acid but no gas. Maltose, lactose and mannitol were not fermented gelatin liquefied. The results were shown in Table I.

In our study the polyherbal plant materials were used to screen the antidandruff activity. The polyherbal formulation was subjected to determine the physical appearance and content analysis. The formulation appears as dark yellow in appearance and found to be clear, viscous and pH ranges 6.3-6.5.

Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents that can be used in the synthesis of useful drugs. The plant's medicinal significance is derived from its bioactive phytochemical components, which have distinct physiological effects on the human body. The polyherbal formulation results were shown in TABLE II. The sterility check was made to prove the purity of the polyherbal extract. The invitro antidandruff activity of polyherbal shows the zone of inhibition as 14 to 33 mm in different concentrations and the chemical shampoos show zones of inhibition. Ketoconazole shampoo shows 14 mm, Himalaya Antidandruff shampoo shows 13 mm, Meera shows 13 mm, Head and shoulders show that 24 mm and Dove shows 21 mm. TABLE III shows the values of the polyherbal shampoos antidandruff zone of inhibition.

However it was reported that the polyherbal extract mixture gave the best result to treat the dandruff because it is natural and easily available with no side effects. It's best treatment for dandruff as well as hairfall control

**TABLE I (A):****Different plant extracts used and their common names**

<b>SCIENTIFIC NAME</b>	<b>PLANTSPARTS USED</b>	<b>EXTRACTED</b>	<b>DOSE USED FOR ANTIFUNGAL ACTIVITY</b>
<i>Acacia concinna</i>	Dried fruit	Water	1:20
<i>Azadirachtaindica</i>	Leaves	Water	1:10
<i>Lawsoniainermis</i>	Leaves	Water	1:10
<i>Hibiscus rosa-sinensis</i>	Leaves	Water	1:5
<i>Citrus limon</i>	Fruit skin peel	Water	1:20
<i>Phyllanthusemblica</i>	Fruit	Water	1:20
<i>Murrayakoenigii</i>	Leaves	Water	1:5
<i>Vigna radiata</i>	Seeds	Water	1:5
<i>Cicer arietinum</i>	Seeds	Water	1:5
<i>Rosa</i>	Flowers	Water	1:5
<i>Trigonellafoeum-graceum</i>	Seeds	Water	1:5

**TABLE I (B):****Active ingredients in different commercially available shampoos**

S.NO	ACTIVE ANTIDANDRUFF INGREDIENTS	SHAMPOOS
1.	Zinc Pyrithione	Head and shoulders
2.	Selenium sulphide	Head and shoulders and dove
3.	Ketoconazole	Antidandruff ketoconazole shampoo

**TABLE II**

S.NO	PHYTOCHEMICALS	POLYHERBAL EXTRACTS
1.	Glycosides	+
2.	Flavonoides	+
3.	Alkaloides	+
4.	Saponin	+
5.	Resin	+
6.	Tannin	+
7.	Steroids	+

**TABLE III**

S.NO	BIOCHEMICAL TEST	POLYHERBALEXTRACTS
1.	Indole	+
2.	Methyl Red	+
3.	VogesProskaver's Test	+
4.	Citrate Utilization Test	+
5.	Oxidase	+
6.	Triple Sugar Agar Test	+
7.	Urease	+
8.	Catalase	+
9.	Esculin Hydrolysis Test	+

**TABLE IV****Zone of inhibition of antidandruff shampoo**

Name of shampoo	Zone of inhibition
Ketoconazole shampoo	14 mm
Himalaya antidandruff shampoo	13 mm
Meera shampoo	13 mm
Head and shoulder shampoo	24 mm
Dove antidandruff shampoo	21 mm

## DISCUSSION

Dandruff is a condition in which white scales of dead skin are shed by the scalp. It develops as a natural process of renewing epidermal cells in the skin of the scalp. It develops as a natural process of renewing epidermal cells in the skin of the scalp. Dandruff is the remainings of dead skin cells that can be replaced by new cells. Yeast like lipophilic basidiomycetes fungus are responsible for dandruff. The shampoos made of herbs entirely could be created that would be superior than those made of synthetic materials.

The present study was to evaluate the antidandruff activity of polyherbal eco-friendly herb care cosmetics with commercial dandruff shampoos.

Lemon juice exhibited the highest level of activity among the chosen plant parts. In addition to lemon extracts, hibiscus leaves showed good efficacy. Neem leaf, Neem fruit, Henna, Amla, Aloe vera, and Shikkai extracts. In comparison to other plant extracts that were created and tested for their synergistic effect against *Malassezia*, lemon, hibiscus leaf, amla, shikakai, and neem demonstrated good antifungal activity. Similar to our study **Dr. Kutcharlapati et al, 2019**, has stated that studying the antidandruff properties of four different antidandruff shampoo brands and noting their zone of inhibitions These findings contrasted the outcomes of the extracts and shampoos and were used as the standard reference. In contrast, it may be said that plant extracts shown significant efficacy against the dandruff-causing organism *Malasseziafarfar* and that these extracts can be used to treat dandruff without creating any negative side effects.

The white cream coloured colony morphology and meat ball shape microscopic appearance shows the *Malassezia* sps, similar to our study **Nagapadma et al, 2015**, denotes that the biochemical studies indicated that fermentation dextrose and xylose produced acid but no gas. Maltose, lactose, rhamnose, raffinose and mannitol were not fermented by *M. furfur*. Liquefaction of gelatin was observed and there was acidification of litmus milk.

The biochemical activity confirmed that the isolated organism as *Malassezia*. As similar to our study, **Vijayakumar R.et al, 2006**, the catalase test was carried out to ascertain the presence of the *Malassezia* species as it is catalase positive, except the *M. restricta* which is catalase negative.

The polyherbal shampoo appears dark yellow colour due to the strong natural ecofriendly skin safe ingredients. Similar to our study, **Chandra et al, 2013**, has obtained that Evaluation of the created product was done using test criteria as pH, % solid contents, viscosity, cleaning action, foam stability, and in vitro antidandruff activity was subjected to evaluation using comparable criteria a commercially available synthetic antidandruff shampoo. For any cosmetic preparation, including shampoo, having a good physical look is always necessary. Physical evaluations were done for qualities including transparency, smell, and colour. The created composition called LF, had a translucent dark brown colour.

The phytochemical analysis shows that the bioactive compounds are responsible for the synthesis of useful drugs. Similar to our study, the phytochemicals like glycosides, Flavonoids, alkaloids, saponin, resin, tannin, steroids shows the positive result.

Similar to our study **Srinivasan prabhu et a, 2022**, has stated that all antidandruff shampoo samples had good antifungal activity but there is a considerable variation in the potency of their antifungal activity depending on the active compound and its concentration.

The antidandruff activity shows that highest zone of inhibition towards *Malassezia* sps. 33 mm, which shows the highest zone of inhibition when compared to synthetic shampoos shows 24 mm. Similar to our study, **Prabha Manju et al, 2012**, denotes that all the antidandruff shampoos had demonstrated strong antifungal efficacy, however the strength of their antifungal activity varied significantly depending on the active ingredient and its concentration. The best anti-dandruff shampoo in the current trial was *vivel ultra pro* because it contains *ketokanazole*, which is known to be an anti-malassezial agent. *Dove* and *Head and shoulders* were then consumed, since they contain antifungal ingredients such as *zinc pyrithione*. The majority of plant extracts had effective antifungal activity that was nearly on par with that of shampoos that are sold in stores. The active ingredients in *lemon*, *henna*, *soap nut*, may have contributed to their greater antifungal action.

The study was carried out to prove that the both commercial cosmetic and the polyherbal shampoos has the antidandruff activity. However, the continuous use of the synthetic shampoo leads to the damage of the scalp and the budding of the hair shaft. As well as in the other side, the polyherbal hair care shampoo prevents the hair fall and gives the constant moisture content to the scalp and also act as an excellent antidandruff agent.

For this investigation the polyherbal herb care cosmetic have the highest efficiency to cure dandruff and eco-friendly and skin safe.

Further studies are carried out to justify the potential of polyherbal for the improvement of antidandruff activity.

## CONCLUSION

The study clearly shows that the anti-dandruff shampoos with *zinc pyrithione* and *piractoneolamine* were far more effective than the other samples. In comparison to herbal antidandruff shampoos, synthetic antidandruff shampoos are dangerous. These shampoos induce transient hair loss as well as scalp burning, skin irritation, and skin peeling. The herbal shampoo is not only safer than using the chemical shampoo, but also significantly lessens the hair loss. Herbal ingredients like *Acacia concinna*, *Azadirachta indica*, *Lawsonia inermis*, *Hibiscus rosa-sinensis*, etc. also demonstrated good antidandruff agent. Herbal cosmetics are becoming more and more popular, as it is widely thought that they are risk free and have no negative side effects. We have both synthetic and herbal shampoos for treating dandruff. Herbal shampoos are often safer and more efficient to use than synthetic anti-dandruff shampoos.

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