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## Formulation and evaluation of anti-dandruff shampoo with synthetic and synthetic agents

**Nayak Rutu, Vyas Jigar and Upadhyay Umesh**

### Abstract

Dandruff is a common ailment affecting the scalp caused by the yeast *Pityrosporum*. Dandruff cannot be totally eradicated; rather, it can be efficiently managed. The presence of pieces, itching of the scalp, and redness around the scalp are the most common dandruff symptoms. There are two techniques to treat dandruff. Chemical antidandruff shampoo and herbal antidandruff shampoo with antibacterial and antifungal components are among them. It is found that synthetic agents having many side effect. To overcome such drawback, shampoo with combination of herbal and synthetic agent are formulated by evaluating their anti-microbial activity via agar diffusion method

**Keywords:** evaluation, anti-dandruff, synthetic

### Introduction

In humans, the scalp is unique and delicate. It has a thick skin layer, high follicular density, and numerous sebaceous glands. The pH of the scalp is 5.5, while the hair shaft pH is 3.67<sup>[1]</sup>. Sebaceous gland on scalp make it prone to different infections including dandruff, parasitic infections such as *Pediculosis capitis*<sup>[2]</sup>. There are various types of skin disorders as follows

1. Alopecia- Hair loss.
2. Seborrheic Dermatitis- inflammation of scalp skin characterized by scaly, itchy, flaky skin.
3. Ring Worm-also known as *Tinea capitis* is a cutaneous fungal infection of scalp, the causative fungi is *Trichophyllum rubrum*.
4. Scalp psoriasis- It's a common scalp skin disorder characterized by raised reddish patches that may spread beyond the scalp to forehead or back of the neck or ears.
5. Scalp folliculitis- Inflammation of hair follicle.
6. Head Lice- scientifically termed as *Pediculosis capitis*, is a contagious infection caused by an obligate parasite, called *Pediculus humanus capitis*, resides on human scalp and feeds on human blood.
7. Dandruff- It is the shedding of the dead skin cells.

### Introduction to dandruff

Dandruff is a widespread scalp condition that affects over half of the population in their prepubertal years, regardless of gender or race<sup>[3]</sup>. The word dandruff (dandruff, dandriffe) comes from Anglo-Saxon, 'tan' meaning 'tetter' and 'drof' meaning 'dirty'. Dandruff has negative impact on appearance and frequently causes itching. Keratinocytes are known to play an important role in the expression and production of immune reactions during dandruff development<sup>[4]</sup>.

### Cause of dandruff

a) Microbial and b) Non-microbial

#### Microbial cause

1. Fungal: *Malassezia furfur* is regarded as the main reason of dandruff
2. Bacterial: the proportions of the two major bacterial communities present on the scalp can differ. Dandruff can also be caused by *Propionibacterium acnes* and *Staphylococcus epidermidis*<sup>[5, 6]</sup>.

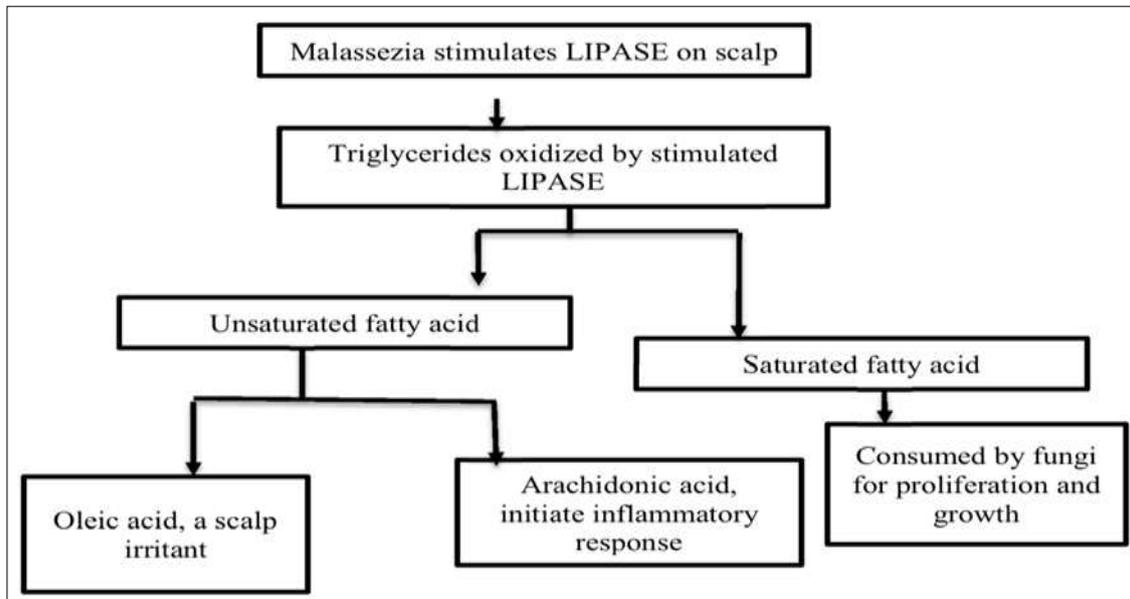


Fig 1: activity of malassezia

#### Non-microbial

1. Corneum stratum [7]
2. Individual vulnerability to oleic acid.
3. Scalp dryness.

#### Dandruff – Pathophysiology

**Fungal colonization** - Malassezia has lipase activity, which hydrolyzes triglycerides in human sebum to produce unsaturated fatty acids (such as oleic and arachidonic acid) [8, 9]. These compounds promote deviant keratinocyte differentiation, which causes anomalies in the stratum corneum such as parakeratosis, intracellular lipid droplets, and uneven corneocyte envelope [10]. Such changes affect epidermal barrier function and trigger an inflammatory response, which may or may not be apparent locally.

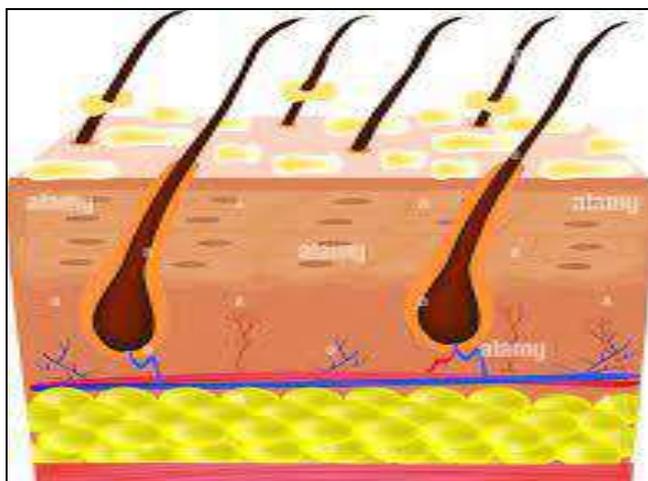


Fig 2: fungal growth

**Sebaceous gland activity** - Sebum synthesis is regulated by hormones, and SGs are activated at birth by maternal androgens acting on androgen receptors in sebocytes [11]. SGs are reactivated during puberty under the direction of circulating androgens [10], resulting in increased sebum secretion during adolescence, which is maintained between the ages of 20 and 30, after which it declines [13]. The secretion rate is higher in males during active sebum

production and continues higher for longer in males between 30 and 60 years of age; in females, the rate reduces rapidly after menopause [14]. SD and dandruff have a strong time link with SG activity, with cradle cap after birth, increased incidence throughout adolescence, increased incidence between the third and sixth decades, and then decreased incidence.

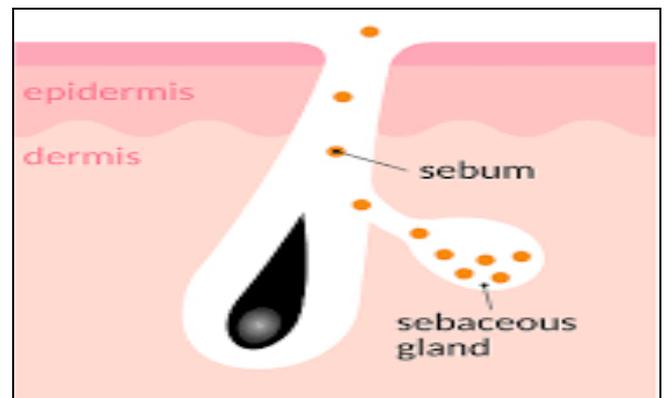


Fig 3: sebaceous gland

**Individual susceptibility:** Other factors, in addition to sebaceous activity and Malassezia colonisation, have a role in the aetiology of SD. Individual susceptibility has been linked to epidermal barrier integrity, host immunological response, neurogenic variables and emotional stress, and dietary factors.

**Epidermal Barrier Integrity:** However, in SD and dandruff, altered corneodesmosomal hydrolysis can affect lipid organisation and the desquamation process, resulting in a barrier function that is abnormal [15]. Barrier structural anomalies such as intercellular Malassezia yeasts, alterations in corneocyte shape and corneodesmosomes, and altered lipid lamellar structure have been found in dandruff scalp by electron microscopy, supporting this theory [16]. In line with the structural findings, dandruff patients have been found to be more reactive to topical administrations of histamine or oleic acid to the scalp (greater itch perception

or flaking) than controls [17]. These findings suggest that a disruption in EPB function may contribute to dandruff exacerbation. Recent genetic investigations in humans and animals reveal that a disruption in barrier function may generate SD-like symptoms directly [18]. In the absence of visible inflammation, biochemical examination revealed that dandruff skin had changed protein profiles, as well as those of SC ceramides and free fatty acids [19]. In the treatment of SD and dandruff, these studies highlight the need of barrier restoration and maintenance.

**Immune response:** Immunosuppression is linked to both the occurrence and severity of SD, especially in HIV/AIDS patients. Because no apparent changes in *Malassezia* levels were detected in this cohort between those with and without SD, it's likely that an immunological or inflammatory response is to blame [20]. Indeed, in one investigation, the human leukocyte antigens HLA-AW30, HLA-AW31, HLA-A32, HLA-B12, and HLA-B18 were reported to be higher in SD [21]. SD patients have also been found to have higher levels of total blood IgA and IgG antibodies [22]. However, no increased antibody titers against *Malassezia* were found, implying that the increased immunoglobulin synthesis is a reaction to yeast metabolites [23]. Infiltration of Natural Killer (NK) cells and macrophages, as well as local complement activation and increased production of inflammatory cytokines such as IL-1, IL-1, IL-6, and TNF- $\alpha$  in afflicted skin areas, are all part of the robust inflammatory response triggered by these compounds [24]. The absence of a rise in anti-*Malassezia* antibodies suggests a shift in the cellular immune response rather than the humoral immune response [25]. The precise significance of lymphocyte activation is still debated [26].

**Genetic factors:** The genetic components of SD and dandruff have been overlooked until recent studies in animal models and people revealed inherited dominant and recessive types of SD and dandruff. A spontaneous mutation in the outbred Him: OF1 mice caused seborrhea, rough coat, baldness, growth retardation, and sometimes aberrant pigmentation in homozygous mutants of the autosomal recessive "inherited seborrheic dermatitis" (seb) mice [27]. In the epidermis and dermis, histological investigation revealed increased sebaceous glands, hyperkeratosis, parakeratosis, acanthosis, and inflammatory infiltrates. There were no yeasts or dermatophytes found. Though the underlying mutation remains unknown, these mice were the first animal model of SD to show a definite route of inheritance [28].

### Symptoms

Dandruff signs and symptoms may include:

- Skin flakes on your scalp, hair, eyebrows, beard or mustache, and shoulders
- Itchy scalp
- Scaly, crusty scalp in infants with cradle cap

The signs and symptoms may be more severe if you're stressed, and they tend to flare in cold, dry seasons.

### Advantage

1. The anti-dandruff shampoo has a relaxing composition that relieves irritation quickly.
2. Reduce itching and redness
3. Patient compliance
4. Flakefree hair

### Treatments available

**Shampoo:** remove greasy residues from previously applied hair care products, such as dirt from the scalp and other environmental pollutants, sebum, perspiration, desquamated corneocytes, and other greasy remnants [29].

**Cream/lotion:** In case of treatment of dandruff, various active agents are formulated as cream, gel or lotion.

**Hair oil:** Oils as hair product are preferred due to moisturizing effect. It reduces drying of scalp and calm the itching. Most of herbal antifungals can be formulated as hair oil

**Tablet:** antifungal drug like itraconazole, Terbinafine HCl and clindamycin Phosphate are used for treatment of dandruff.

### Materials and method

**Herbal antifungal agents used:** Garlic extract, Onion extract, Piper, Eucalyptus oil, Tea tree oil

**Synthetic antifungal agents used:** Sertaconazole Nitrate, Ketoconazole, Zinc PTO, Ciclopirox olamine

### Garlic extract

**Source** – Obtained from bulb *Allium Sativum*

**Family** – Liliaceae

**Mechanism of action** - Garlic contains alliin, ajoene, fructosans, and saponins. 4 An alliin, diallyl thiosulfinate, is thought to be responsible for garlic's antibacterial and antifungal properties. 5 In China, another alliin, diallyl trisulfide, is used for the treatment of bacterial and fungal infections. Garlic intake in humans has been reported to enhance natural killer cell activity and immune activity.

### Onion

**Source** – Obtained from bulb *Allium Sativum*

**Family** – Liliaceae

**Mechanism of action** - Onion is known for its antibacterial properties, and hence, it is helpful in getting rid of dandruff and an itchy scalp. onions contain Allicin, which is known for its antibacterial properties. compound is powerful protection against germs.

### Piper

**Source** – Obtained from fruits of *Piper nigrum*, the source plant of both black and white pepper

**Family** – Piperaceae

**Mechanism of action** – study says that piper has activity equivalent to ketoconazole. It functions by preventing the synthesis of ergosterol, the fungal equivalent of cholesterol, thereby increasing membrane fluidity and preventing growth of the fungus.

### Tea tree extract

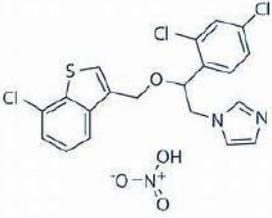
**Source** - obtained from *Melaleuca alternifolia*

**Family – Myrtaceae**

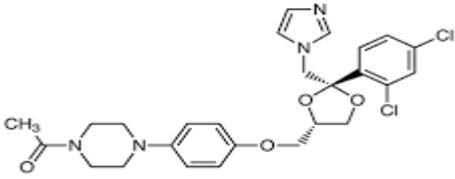
**Mechanism of action** - The components of tea tree oil, particularly terpinen-4-ol and  $\alpha$ -terpineol, mediate

antimicrobial actions by disrupting the structural and functional integrity of bacterial membrane.

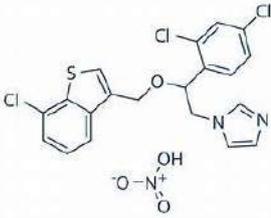
**Sertaconazole Nitrate**

|                     |   |
|---------------------|---|
| Category            | Imidazole / triazole type antifungal agent  |
| Chemical structure  |  The chemical structure of Sertaconazole Nitrate consists of a 2-chlorophenyl-5-thiazolylmethyl ether group attached to a 2-(2,4-dichlorophenyl)ethyl group, which is further linked to an imidazole ring. A separate nitrate ion (NO3-) is shown below the main structure. |
| Mechanism of action | Sertaconazole, an azole antifungal agent, inhibits fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylase enzyme. This enzyme functions to convert lanosterol to ergosterol   |

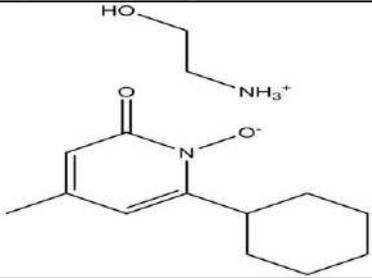
**Ketoconazole**

|                     |  |
|---------------------|--|
| Category            | Imidazole type antifungal agent  |
| Chemical structure  |  The chemical structure of Ketoconazole features a piperazine ring with a methyl ketone group on one nitrogen and a 4-(2-(2,4-dichlorophenyl)ethoxy)phenyl group on the other. This is connected to a 2-(2,4-dichlorophenyl)ethyl group, which is further linked to an imidazole ring. |
| Mechanism of action | It functions by preventing the synthesis of ergosterol, the fungal equivalent of cholesterol, thereby increasing membrane fluidity and preventing growth of the fungus.[30]  |

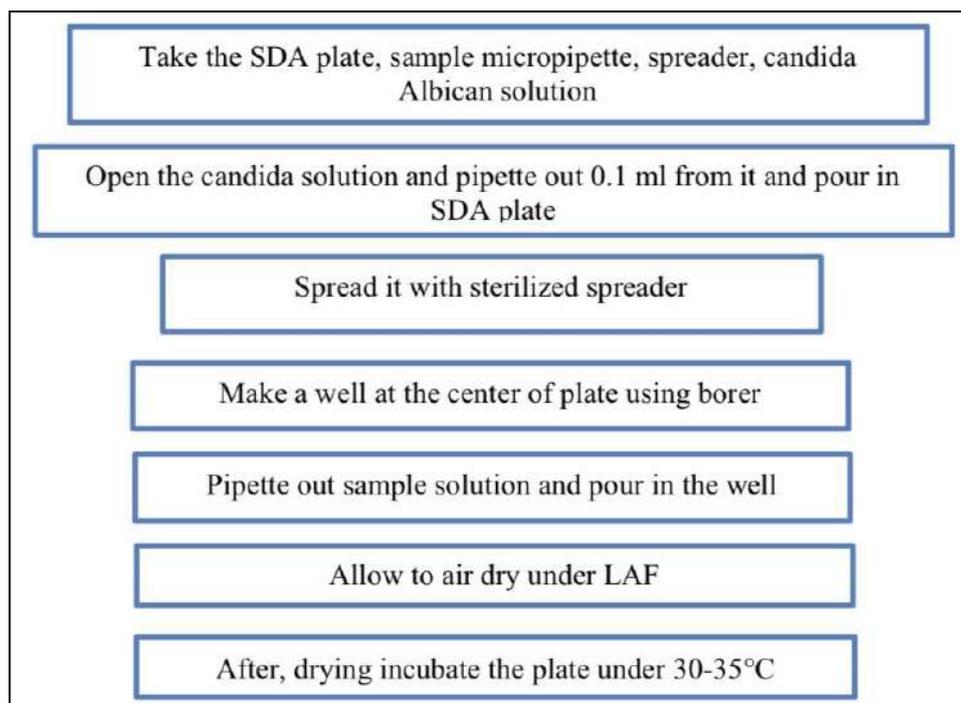
**Zinc PTO**

|                     |   |
|---------------------|---|
| Category            | Coordination complex of zinc. It has fungistatic  |
| Chemical structure  |  The chemical structure of Zinc Pyrithione is a coordination complex where a zinc atom is coordinated to two thiazole rings and two pyridine rings. A nitrate ion (NO3-) is shown below the main structure. |
| Mechanism of action | Zinc pyrithione (or pyrithione zinc) is a coordination complex of zinc. It has fungistatic (that is, it inhibits the division of fungal cells) and bacteriostatic (inhibits bacterial cell division) properties and is used in the treatment of seborrhoeic dermatitis.                         |

**Ciclopirox olamine**

|                     |  |
|---------------------|--|
| Category            | A cyclohexane and pyridinone derivative  |
| Chemical structure  |  The chemical structure of Ciclopirox olamine consists of a pyridinone ring system with a methyl group at the 2-position and a cyclohexane ring at the 4-position. A 2-aminoethanol group is attached to the nitrogen atom of the pyridinone ring. |
| Mechanism of action | It causes membrane instability by accumulating inside fungal cells and interfering with amino acid transport across the fungal cell membrane.  |

## 2.1 Method to perform microbial assay by agar diffusion:



**Fig 4:** procedure for microbial assay

### pH and pH stability

Before you begin to calibrate and use your pH meter you will first need to turn it on and allow adequate time for the meter to warm up. This should generally take around 30 minutes, but check your pH meter's operating manual for exact times. Take the electrode out of its storage solution and rinse it with distilled water under an empty waste beaker<sup>[31]</sup>. You will generally need more than one buffer for calibrating a pH meter. The first will be a "neutral" buffer with a pH of 7, and the second should be near the expected sample pH of 4.<sup>[32]</sup> Once you have a stable reading, set the pH meter to the value of the buffer's pH by pressing the measure button a second time. Setting the pH meter once the reading has stabilized will allow for more accurate and tuned readings. Press the measure button to begin reading the pH once your electrode is placed in the buffer. Once your electrode is placed in your sample, press the measure button and leave the electrode in your sample for approximately 1-2 minutes. Once the reading has stabilized, press the measure button. This is the pH level of your sample<sup>[33]</sup>. Most shampoos are neutral or slightly acidic. Acidic solutions cause the cuticle (outer layer) of the hair to shrink and lie flatter on the hair shaft. Basic solutions cause the cuticle to swell and open up. Acidic solutions make the hair smoother, while basic solutions make the hair frizzier.

### Viscosity

Viscosity of the formulated gels was determined by using Brookfield viscometer (Brookfield DV-II+ Pro). The samples to be analyzed were taken in a 25 ml beaker. The viscosity of the samples was measured by using spindle number 96. The test was repeated 6 times for each sample and the average value was calculated. The test was conducted at room temperature and angular velocity was kept at 10 rpm<sup>[34]</sup>.

### Physical appearance

Physical appearance of the formulated shampoo was evaluated by visual inspection. Parameters such as consistency, color and transparency was analyzed<sup>[35]</sup>.

### Foaming capacity and foam stability

The cylinder shake method is the most widely used method for determining foaming ability<sup>[36]</sup>. At room temperature, 1% of 50 mL of the shampoo solution was put into a 250-mL graduated cylinder, which was then covered by hand and shaken ten times. The total volume of the foam content after 1.0 min of shaking was recorded. The height of the foam generated was measured immediately. To evaluate foam stability, the same procedure was performed and the foam volume after 20 min was measured.

### Solid content

Evaporating plate of known plate are taken and 4 gm of shampoo was added to it. Dish is allowed to heat on hot plate until liquid portion get evaporate and quantity of solid content is calculated.

### Experimental work

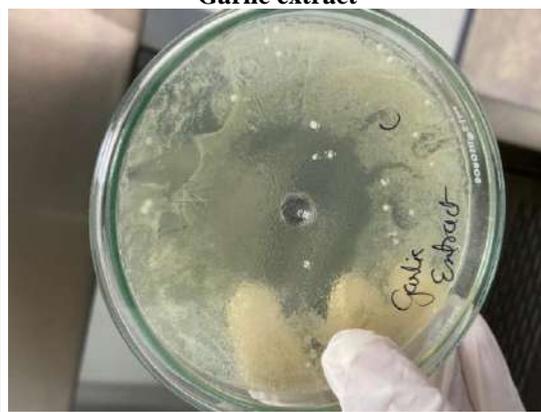
#### Assay of herbal and synthetic drugs

| Ingredient            | F1  | F2  | F3  | F4  | F5  | F6  | F7  | F8  | F9  |
|-----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Onion                 | 1.0 |     |     |     |     |     |     |     |     |
| Garlic                |     | 1.0 |     |     |     |     |     |     |     |
| Eucalyptus oil        |     |     | 1.0 |     |     |     |     |     |     |
| Piper                 |     |     |     | 1.0 |     |     |     |     |     |
| Tea Tree Extract      |     |     |     |     | 1.0 |     |     |     |     |
| Sertaconazole nitrate |     |     |     |     |     | 2.0 |     |     |     |
| Zinc PTO              |     |     |     |     |     |     | 1.0 |     |     |
| Ketoconazole          |     |     |     |     |     |     |     | 2.0 |     |
| Cicloperoxolamine     |     |     |     |     |     |     |     |     | 1.0 |

**Onion extract**



**Garlic extract**



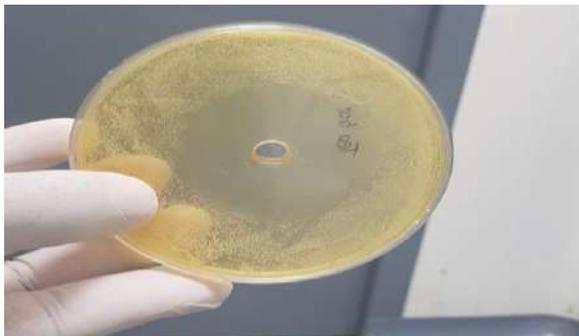
**Eucalyptus oil**



**Piper**



**Tea tree oil**



**Ciclopirox olamine**



**Sertaconazole nitrate**



**Zinc PTO**



**Ketoconazole**



From above result, ciclopirox olamine and tea tree oil is shown to have highest efficiency against *Candida Albican*. Formulaion will be designed as purely synthetic, purely

herbal and combination of synthetic herbal anti-dandruff agents. Reduction of synthetic agent will be carried out with use of herbal agent, as it gives synergetic effect

## Formulation of shampoo

| Material                        | F1    | F2    | F3    |
|---------------------------------|-------|-------|-------|
| Ciclopirox olamine              | 1.00  | -     | 0.5   |
| Tea tree oil                    | -     | 1.0   | 1.0   |
| Sodium lauryl ether sulfate     | 20.00 | 20.00 | 20.00 |
| Disodium laureth sulfosuccinate | 32.00 | 32.00 | 32.00 |
| Cocoamido propyl betain         | 4.00  | 4.00  | 4.00  |
| Polyquaternium 7                | 2.00  | 2.00  | 2.00  |
| Sodium chloride                 | 1.00  | 1.00  | 1.00  |
| Citric acid                     | 0.20  | 0.20  | 0.20  |
| Purified water                  | 39.8  | 39.8  | 39.8  |
| Total                           | 100   | 100   | 100   |

## Method of preparation of shampoo

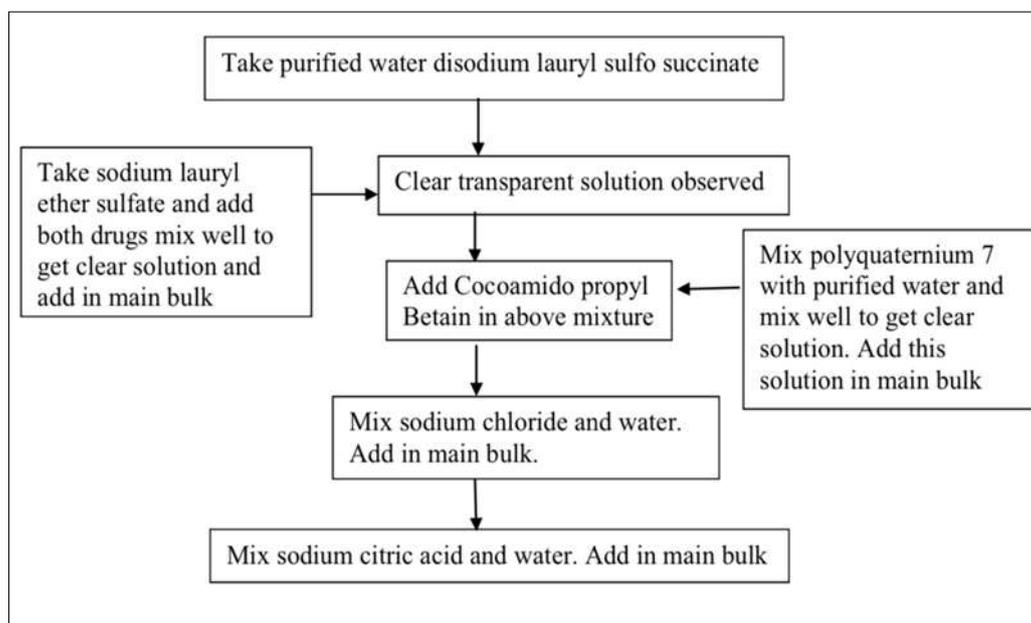


Fig 5: preparation of shampoo

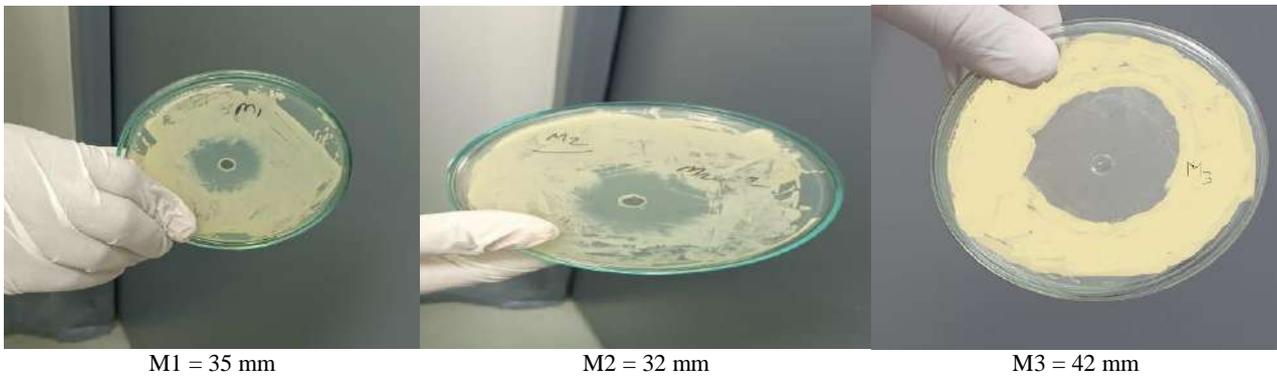
## Result and discussion

### Microbial assay of synthetic and herbal anti-dandruff agents

| Anti-dandruff agents  | Zone of inhibition (in mm) |
|-----------------------|----------------------------|
| Onion extract         | 29 mm                      |
| Garlic extract        | 24 mm                      |
| Eucalyptus oil        | 10 mm                      |
| Piper                 | 21 mm                      |
| Tea tree oil          | 31 mm                      |
| Sertaconazole nitrate | 28 mm                      |
| Ketoconazole          | 25 mm                      |
| Zinc PTO              | 38 mm                      |
| Ciclopirox olamine    | 38 mm                      |

## Evaluation of shampoo

| Parameter              | M1                    | M2                    | M3                    |
|------------------------|-----------------------|-----------------------|-----------------------|
| <b>Microbial assay</b> |                       |                       |                       |
| Appearance             | Clear and transparent | Clear and transparent | Clear and transparent |
| pH                     | 5.74                  | 5.88                  | 5.39                  |
| Viscosity              | 4052                  | 3965                  | 4087                  |
| Solid content %        | 4.5%                  | 6.1%                  | 3.8%                  |
| Foam stability         | 120 ml                | 100 ml                | 105 ml                |



M1 = 35 mm

M2 = 32 mm

M3 = 42 mm

### Conclusion

Dandruff is a scalp condition that affects more than 50% of the human population and affects the social behaviour of the sufferer along with possessing an unhealthy scalp. Novel formulations containing proven anti-dandruff agents with longer contact time at the site of action are the need of the hour and hence can prove to be advantageous in anti-dandruff treatments.

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