

**A comparative study of commercially available shampoos for its anti-dandruff activity**

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**Abstract**

Anti-dandruff shampoos like synthetic or herbal are marketed to combat the problem of dandruff. To test the effect of synthetic and herbal shampoos on dandruff causing fungus to isolate the species *Malassezia*. The fungal species was isolated using Potato Dextrose Agar medium. Total six synthetic and herbal shampoos were selected to check the effectively against dandruff by agar cup method. Different level (0.1 ml, 0.5 ml, 1ml) of shampoos were taken to check the Zone of Inhibition. The selected shampoos were effective as they showed inhibition against dandruff. Synthetic shampoos were more effective when compared to herbal shampoos. The highest zone of inhibition was obtained by Head & Shoulders > Pure derm > All clear and Dove. Herbal anti-dandruff shampoos were found to be an effective but their anti-dandruff activity was less compared to synthetic

one. Currently, herbal hair is high demand for strengthen for negligible side effects.

**Keywords:** *Malassezia*, Synthetic, Dandruff, Shampoo, Scalp.

**Introduction**

Dandruff is a common scalp condition that can affect anyone and it is a huge problem found in people who exposed to dust and other pollutants in day to day life, 5% of the population are affected from dandruff after puberty in the range of 20- 30 years, it is a common scalp disorder and also for a cosmetic problem as it leads to hair fall, and dandruff affects in males than the females. Excessive shedding of skin cells and flaking from the scalp is the reason for causing the dandruff [1-3] for Seborrheic dermatitis, Psoriasis, fungal infection or excoriation are associated with infestation of head lice. The pathogenesis of dandruff involves hyperproliferation, resulting in deregulation of keratinization. Inflammation on scalp skin

along with the appearance of oily scales of dirty yellow color that can form lesions. These lesions can be associated with pruritis of different intensity. The most affected area of dandruff is scalp, but it also occurs between eyebrows, along the side of nose, behind the ears, over the breastbone and sometimes in the armpits [4].

Many factors are responsible for the dandruff such as fungal infection, hormonal deficiency, weather conditions, poor hygienic, strain, anxiety, excessive use of hair spray, gels, alkaline soaps, share combs, hair brushes, inadequate rinsing of hair and irregular shampooing [5]. Adequate exposure of sunlight is also the reason for dandruff, it is known as desquamation of the scalp [6]. Dandruff usually occurs as small, round, white to grey patches on the top of the head. It is not a serious problem but make you feel socially uncomfortable and mortified. It is not a spreadable disease and can be controlled. Dandruff is normal for skin cells to die and flake off is common. Depending on the severity the symptoms can vary from mild to severe. It is determined by the flakes on the scalp [7].

*Malassezia furfur* is a type of fungus belongs to the *Malassezia* species. It is the causative organism for dandruff [8-10]. *Malassezia* is a lipophilic, dimorphic fungus occurs in the human skin cause superficial and deep mycoses such as Pityriasis versicolor, Seborrheic dermatitis [11,12] Being an opportunistic pathogen it causes a disease such as dandruff. *Malassezia* species formerly known as *Pityrosporum* [13]. Dandruff medically known as Pityriasis capitis is caused by *Malassezia furfur*, *Malassezia globosa* and *Malassezia restricta* [14,15].

Today, many treatment options are available for the control of dandruff includes therapeutic use of antidandruff shampoos containing Miconazole, Ketoconazole, Keratolytics, antimicrobials like Zinc

pyrithione, selenium sulphide, salicylic acid, Imidazole derivatives,

- Fungicidal substance - Eg. (Pyrithione andimidazoles)
- Cytostatic substance - Eg. (Tar, selenium sulphide and octopirox)
- Keratolytic substances- Eg. Salicylic acid and sulphur compounds) (Adamsk) [24]. • Synthetic anti-dandruffshampoo

Herbal anti-dandruff shampoo

sulphur and tar derivatives [16]. They have certain limitations because poor clinical efficacy or due to acquiescence issues. In market the number of anti-dandruff shampoos is readily available in various forms such as powder, clear liquid, lotions, solid gel, medicated and liquid herbal shampoo [17-19]. Based on the type of ingredients used, it may be simple shampoo, anti-dandruff shampoo, anti-septic shampoo, shampoo containing vitamins, amino acid and protein hydrolysate called as a nutritional shampoo. It may require for the washing of hair and scalp packed in a form which is fitting for use they may be anionic, cationic, and non- ionic surfactants [20,21].

The gentle shampoos to reduce oiliness and mild dandruff but regular shampoos are not valid in dandruff, so they are used antidandruff shampoos, but all the antidandruff shampoos are not alike. Shampoo is hair care product the primary role of shampoo is rinsing or detergent action, lubrication, conditioning, prevention of static charge gradually builds up in hair, but the removal of dandruff also one of the important characteristics of a good shampoo, the complete formulation of shampoos is must be medically safe for long term usage is important for the hairy region.21

In Worldwide, so many traditional systems are available, practiced and prove that throughout the areas including Ayurvedic medicine, Unani, and Traditional Chinese

Medicine can be used as medicine and maintaining good health [22]. They also represent an excellent source of secondary metabolites for growing food additives and pharmaceuticals industries. The demand for natural products are highly increased in Worldwide. The use of medicinal plants and cosmetic purposes is inextricably linked to ancient and modern cultures of Asian countries. Skin is outer covering and protecting of the human body that provides the environment from unfavorable external factors [23].

Depending on the formulation shampoos, oils, creams, lotions, and other cosmetic products are available in the market. These formulations include therapeutic use of antidandruff agents that are classified into three groups according to their mechanism of actions.

Two types of antidandruff shampoos are available commercially, The word herbal or Ayurveda is a symbol of safety when compare to synthetic one which has adverse effects on human health. In Ayurveda, the several plants are useful effectively against various causes of dandruff [25]. Today the plants have a major part that involve in skincare and cosmetic, WHO estimates that up to 80% of the people used natural products.

#### Commonly used plants in herbal shampoos

| S. No | Name of the plants     |
|-------|------------------------|
| 1     | Piper betle            |
| 2     | Hibiscus rosa-sinensis |
| 3     | Datura metal           |
| 4     | Citrus aurantifolia    |
| 5     | Camellia sinensis      |
| 6     | Ficus bengalensis      |
| 7     | Lawsonia inermis       |
| 8     | Phyllanthus emblica    |
| 9     | Ocimum sanctum         |
| 10    | Zingiber officinalis   |

|    |                         |
|----|-------------------------|
| 11 | Azadiracta indica       |
| 12 | Aloe vera               |
| 13 | Mangifera indica        |
| 14 | Murraya koenigii        |
| 15 | Mentha asiatica         |
| 16 | Nigellasativa           |
| 17 | Santalum album          |
| 18 | Sapindus mukorossi      |
| 19 | Nyctanthes arbortristis |
| 20 | Vetiveria zizanioides   |
| 21 | Vitex negundo           |

Herbal shampoos are the cosmetic preparations with the use of Ayurvedic herbs which contains herbal ingredients such as plant extract and essentials oils. Now-a-days natural products are useful over the synthetic products because there are a no side effects. This is the reason; herbal products are popular among the consumer [26]. Most of the plants which are reported to have beneficial effects on hair for removal of oils, dandruff, dirt and environmental pollutions. The best approach to treat dandruff is to use plants and herbal formulations which possess anti dandruff properties.

#### Isolation of fungal species

**Sample collection:** Flakes were collected from scalp by partitioning the hair with a sterile comb and scrapping approximately one-inch area using a sterile blunt scalpel. The specimen was then transferred into dark sampling paper to prevent exposure to sunlight. The samples were inoculated in the Potato Dextrose Agar plus olive oil which was incorporated with chloramphenicol to avoid bacterial contaminants into sterile Petri plates. The plates were incubated at 30°C for 3 days and observed regularly.

**Sample Analysis 2.3.1. Direct microscopy:** A drop of 10 % potassium hydroxide was added onto a clean slide which contains the smear of sample and cover using a

coverslip. The sample was then heated over a Bunsen burner to remove bubbles. The slides were viewed under 40X magnification [27,28].

**Culture:** The collected samples were cultured on PDA medium which was incorporated with chloramphenicol to get rid of the bacterial contaminants. Small amount of the samples collected were introduced into petri dishes containing media using sterile forceps. The petri plates were labelled appropriately and incubated at 30°C for 2days.

**Catalase test:** Catalasetest was carried out to confirm the presence of fungal species to test whether it is positive or negative. 3 ml of 3 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution was

## Materials and Methods

### Biochemical test

**Catalase test:** Catalasetest was carried out to confirm the presence of fungal species to test whether it is positive or negative. 3 ml of 3 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution was poured into a test tube. T - the isolated fungal colonies were immersed into the test tube using a sterile glassrod.

**Agar Cup Method:** ACM was performed to check the antifungal activities of shampoo. Two days' prior small amount of culture was inoculated into Potato Dextrose Agar was used to prepare plates was maintained for this assay. Each plate contained a well of 0.6 cm in diameter the different shampoos were added to the well using pipette. Experiments were done using suitable controls.

### Anti-fungal Activity

**Agar Cup Method:** ACM was performed to check the antifungal activities of shampoo. Two days' prior small amount of culture was inoculated into Potato Dextrose Agar was used to prepare plates was maintained for this assay. Each plate contained a well of 0.6 cm in diameter

the different shampoos were added to the well using pipette. Experiments were done using suitable controls.

**Table 1: List of Anti-dandruff shampoos used during the experiment work**

| S.No | Name of Anti-dandruff shampoo | Active Ingredients     | Manufacturer               |
|------|-------------------------------|------------------------|----------------------------|
| 1    | Head & Shoulders              | Zinc Pyrithione        | Procter & Gamble           |
| 2    | All Clear                     | Zinc Pyrithione        | Hindustan Unilever         |
| 3    | Himalaya                      | Tree Tea Oil           | Himalaya Herbs Health care |
| 4    | Dove                          | Zinc Pyrithione        | Hindustan Unilever         |
| 5    | Pure derm                     | Oxy fused micro bubble | Hindustan Unilever         |
| 6    | Ayush                         | Rose marry oil         | Lever Ayush                |
| 7    | Panjanli                      | Hibiscus, Biringarag   | OEM Manufacture            |
| 8    | Karthika shikakai             | Hibiscus, Shikakai     | Cavinkare                  |
| 9    | Meera shikakai                | Green gram, Thulsi     | Cavinkare                  |
| 10   | Homemade shikakai             | Hibiscus, shikakai     | Home                       |
| 11   | Karthika shampoo              | Hibiscus, Fenugreek    | Cavinkare                  |
| 12   | Meera shampoo                 | Sodium lauryl sulphate | Cavinkare                  |

### Zone of Inhibition (ZOI)

ZOI was done on PDA plates by agar cup method before two days' active culture was inoculated into the PDA medium. All the shampoos were dissolved in 9 ml sterile distilled water. The same procedure is done to check the zone of inhibition by incubating at 30°C for 24hours. After incubation, the plates were observed. The zone of inhibition was measured using a zone measuring scale and the results were observed.

### Statistical analysis

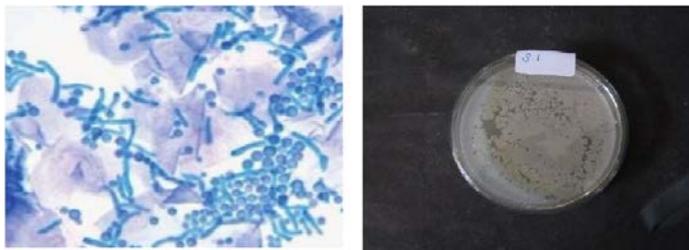
All the analyses were calculated using the Statistical Package of Social Sciences (SPSS) software package version 16.0. Results with P < 0.05 were considered to be statistically significant.

### Results

**Isolation of fungal species:** The collected samples were streaked over the surface of Potato Dextrose Agar and 3 different colonies were obtained. Further, these colonies were individually streaked in separate potato dextrose agar plates. According to the morphological features,

Malassezia was identified and then inoculated in PDA medium. The sample was analyzed on the basis of direct microscopy of the collected sample of scalp containing dandruff ovoid shaped cells.

Figure 1: Isolation of fungal species from the Potato Dextrose Agar plates



**Biochemical test:** Catalase test was performed which showed active bubbling, the isolated colonies of fungi were inoculated in a test tube containing 3 ml of 3 % H<sub>2</sub>O<sub>2</sub>, to indicating positive result.

Figure 2: Catalase test (anti-fungal activity)



### Anti-fungal Activity

**Agar Cup Method:** Agar cup method was performed throughout the experiment for different samples (Shampoos) to check the inhibition levels of dandruff.

Table 2: Shampoos and its Zone of Inhibition

| Name of shampoo   | Zone of Inhibition in diameter (mm) |
|-------------------|-------------------------------------|
| Head & Shoulders  | 2                                   |
| All Clear         | 2.3                                 |
| Dove              | 1.8                                 |
| Pure derm         | 2.3                                 |
| Homemade shikakai | 1.3                                 |
| Karthika shikakai | 0.4                                 |
| Meera shikakai    | 0.4                                 |

Figure 3: Preliminary test of anti-fungal activity



**Zone of Inhibition (ZOI):** Zone of Inhibition was performed with different levels of anti-dandruff shampoos and to check efficiency of shampoos in terms of inhibition.

Figure 4: Synthetic and herbal based shampoos were tested



Figure 5: Confirmatory test of anti-dandruff activity using different shampoos



Table: 3 Measurement of their zone of different shampoos

| S.No | Name of shampoo   | 0.1 ml | 0.5 ml | 1 ml   |
|------|-------------------|--------|--------|--------|
| 1    | Head & Shoulders  | 1.5 mm | 1.9 mm | 2.3 mm |
| 2    | All Clear         | 1.8 mm | 2 mm   | 2.4 mm |
| 3    | Himalaya          | 0.1 mm | 0.5 mm | 1.8 mm |
| 4    | Dove              | 1.3 mm | 1.8 mm | 2.2 mm |
| 5    | Pure derm         | 1.6 mm | 2 mm   | 2.7 mm |
| 6    | Ayush             | –      | 0.3 mm | 0.5 mm |
| 7    | Pantanjali        | –      | 0.1 mm | 0.3 mm |
| 8    | Karthika shikakai | –      | 0.1 mm | 0.2 mm |
| 9    | Meera shikakai    | –      | 0.2 mm | 0.3 mm |
| 10   | Homemade shikakai | –      | 0.6 mm | 1 mm   |
| 11   | Karthika shampoo  | –      | –      | 0.3 mm |
| 12   | Meera shampoo     | –      | –      | 0.2 mm |

### Discussion

Plant extract shows moderate zone of inhibition and local herbal shampoo shows potent antifungal activity, due to the presence of Shikakai, *Aloe vera*, Methi, Soap nuts and *Amla* Prakruthivanam shampoo [29]. But, our study reveals that the synthetic shampoos show more antifungal activity when compare to natural hair wash. Herbal extracts area valid alternative for the chemical preparations. Herbal shampoos and poly herbal hair oils

have excellent results due to their synergistic, antifungal, anti-inflammatory & immuno-stimulatory action. In the present study, we reveal that the zone of inhibition was find more in pure derm, Head & shoulders etc., whereas in natural products the zone of inhibition was less. All the ingredients used to formulate shampoo are safer than silicones and polyquaterniums to synthetic conditioning agents. Instead of using cationic conditioners, shikakai, hibiscus reetha, and various plant extracts have conditioning effect. The formulation of anti-dandruff hair shampoo provides a method for treating a scalp dandruff or seborrheic dermatitis. Polyherbal anti-dandruff hair shampoo containing different concentrations of herbal extract of pomegranate, reetha, shikakai, orange peel, licorice, curry leaves, neem leaves, and hibiscus flower used as an effective agent. Pomegranate is the key ingredient for *M. furfur* species as antifungal. It shows good antifungal effect in combination with increased concentration [30]. In our study we also did the biochemical test such as catalase to know the active bubbling, but we got the good result in synthetic shampoos due to the presence of ketacozinole. The *C. crenata* shell and oil-soluble Glycyrrhiza extracts shows high anti-Malassezia activity and used in the treatment of *Seborrheic dermatitis*. The extracts also showed fungi static activity against other common facultative pathogenic yeasts, *Cryptococcus* and *Candida* [31]. The current study reveals that we did a comparative study for both natural and synthetic shampoos available in market. We got a moderate result in natural products when compare to synthetic ones. Plant extracts showed better activity against dandruff causing organism *Malassezia furfur*. From these results, we came to the conclusion, that plant extracts have antifungal activity and could be safely used for treating dandruff. Synthetic drugs are unable to prevent recurrence [32]. In our study, we didn't use any

plant extract, we used the home-made shikakai and also commercially available powder. But we got a promising result in synthetic shampoos. The study was significant and efficient known plant products with anti-dandruff activity could be compared with commercially available shampoos but also their good efficacies at minimum concentrations could be identified. This can help make a polyherbal mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity [33]. Our study also reveals that we got better result in shikakai in minimum concentration but good result in shampoos [34]. The shampoos are mainly responsible for improving and enhancing hair growth, quality, minimizing eye irritation and other factors.

### Conclusion

Medicinal plants are strong evidenced usage of various secondary metabolites. Therefore, the plants can be explored and studied further for their therapeutic utility. Anti-Dandruff shampoos are widely used for removal of dandruff. All the antidandruff shampoos had better antifungal activity but there are considerable variations in the potency of their antifungal activity depending on the active compound. The formulation contains therapeutic use of anti-dandruff agents such as Fungicidal substances, Cytostatic substances and Keratolytic substances. Herbal shampoos could be used to combat dandruffs and their efficacy was not questionable. Antifungal activity against dandruff with Zone of inhibition ranging from 1 ml, 0.5 ml and 0.1ml. The highest zone of inhibition was observed by All clear, pure derm, Head & shoulder and Dove. Comparatively the synthetic shampoos showed a high zone of inhibition than the herbal shampoos. Herbal anti-dandruff shampoos were found to be an effective but their anti-dandruff activity was less compared to synthetic one.

The usage of various medicinal plants in herbal formulations has to promote the hair growth. The medicinal plants are used and treatment with many herb based medications is progressive in the field. Based on the above results indicated that all the tested shampoos they chemically strong. Due to slight modifications or differences between brands of various manufacturing processes, laboratory conditions and other reasons. Herbal based shampoos are more effective, safety and easily manufacturing and its economic value.

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