ORIGINAL ARTICLE

Development of Polyherbal Shampoo for Antifungal Activity and its Comparison with Commercially Available Shampoo: A Research Article

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Abstract

Background: The study aimed to formulate a pure herbal shampoo, evaluate, and compare its physicochemical properties with the marketed synthetic and marked herbal shampoos. **Materials and Methods:** The herbal shampoo formulated by adding the extracts of different herbs such as pomegranate, neem, hibiscus, shikakai, reetha, curry leaves, licorice, and orange peel. Small amount of methylparaben has been added as a preservative and pH adjusted with citric acid. Shampoo formulations were tested against fungus *Malassezia furfur* by agar well diffusion method. **Results:** The formulated herbal shampoo was clear and appealing. It showed good cleansing and detergency, low surface tension, small bubble size, and good foam stability after 5 min. The prepared shampoo and commercial shampoos showed comparable results for percent solid contents also. **Conclusion:** The results indicated that the formulated shampoo is having excellent, at par with commercially available shampoo. However, further, research and development are required to improve its quality and safety.

Key words: Antifungal activity, dandruff, herbal shampoo, *Malassezia furfur*, marketed shampoo, shampoo formulation

INTRODUCTION

Hairs are the integral part of human beauty. People are using herbs for cleaning, beautifying, and managing hair since the ancient era. As the time has passed, synthetic agents have taken a large share, but today people are getting aware of their harmful effects on hairs, skin, and eyes. These reasons attracted to community toward the herbal products, which are less expensive and have negligible side effects. The primary function of the shampoo is the cleansing or detergent action, but the removal of dandruff also one of the important characteristics of a good shampoo.[1] *Malassezia* species formerly known as *Pityrosporum* is a lipophilic, dimorphic opportunistic yeast causing skin and hair infections such as *Pityriasis versicolor*, seborrheic dermatitis, and dandruff. Dandruff medically described as *Pityriasis capitis* is caused by *Malassezia* species such as *Malassezia furfur*, *M. globosa, and Malassezia restricta.*[2,3] The word herbal signifies a symbol of safety in contrast to the synthetic one which has adverse effects on human health, today's busy life schedule has created the negligence of an individual to protect their hair and maintain the hair homeostasis so there is a need to derive formulations for avoiding hair damages that contain herbal extracts.[4] Shampoos are not only used for cleansing hair but also used to maintain the hair - soft, shiny, thicker, and longer and to remove the oiliness from the hair.

Various forms available such as powder, clear liquid, lotion, solid gel, medicated, and liquid herbal shampoos.[5,6] Depending on the type nature of ingredients used, it may be simple shampoo, anti-dandruff shampoo, and antiseptic shampoo and shampoos containing vitamins, amino acids, and protein hydrolysate called as nutritional shampoo.[7]

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MATERIALS AND METHODS

Collection of plant material

Fresh curry leaves, neem leaves, and hibiscus flower were collected from the local garden of Mandsaur (M.P.). Pomegranate, reetha, shikakai, orange, and licorice were procured from local market of Mandsaur (M.P.). The plant was authenticated by Dr. S. N. Mishra (Head AINP on M and AP) KNK College of Horticulture. Fungal strain was procured from MTCC (Microbial Type Culture Collection Centre and Gene Bank) Chandigarh, India. The fungal strains were cultured on Dixon agar medium at 28°C.

Preparation of crude extracts

Punica granatum L., *Azadirachta indica*, *Murraya koenigii*, *Hibiscus rosa-sinensis*, *Sapindus mukorossi, Acacia concinna*, *Glycyrrhiza glabra,* and *Citrus sinensis* conditioning antimicrobial and antifungal properties already reported were homogenized and extracted using water as a solvent. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (50–55°C) to obtain solvent-free extracts were washed, weighed, and packed into plastic containers and stored in room temperature.

Preparation of polyherbal shampoo

The plant extracts were mixed in different proportions to obtain a shampoo whose formula is shown in Table 1. Herbal extracts were mixed with sufficient quantity of water in one part and triethanolamine lauryl sulfate was mixed with other parts of water after that methylparaben was also added with stirring. Finally, the pH of the solution was adjusted by adding sufficient quantity of 1.5% citric sinensis solution. Few drops of rose essential oil were also added to impart aroma to the prepared shampoo, and the final volume was made to 50 mL with water solution.[8]

Evaluation of polyherbal shampoo

The prepared formulated shampoo was following the subjected parameters.

Physical evaluation

Following physical evaluations has been done for the formulation:

1. pH
2. Foaming capacity
3. Viscosity
4. Stability
5. Detergency, etc.

Visual inspection/physical appearance of shampoo

The appearance or physical inspection of shampoo concern mainly about clarity of shampoo (transparency), color of content, odor, and total foam producing ability.[9]

Determination of pH

About 10% volume by volume shampoo solution in water (i.e., distilled) measured by Ph meter condition: Room temperature.[10]

Rheological evaluations

The viscosity of the shampoos was determined using Brookfield's viscometer.

Foam and foam stability

Ross-miles foam column is an accepted method for measuring foam light and foam stability in this method 200 mL of a shampoo solution fall through an orifice into a glass column

Table 1: Formula for shampoo preparation

Particulars	Part used	Quantity (50 mL)					
		F1	F2	F3	F4	F5	F6
Punica granatum	Fruit	0.25	0.5	1	1.5	2	2.5
Azadirachta indica	Leaves	0.25	0.5	1	1.5	2	2.5
Murraya koenigii	Leaves	0.25	0.5	1	1.5	2	2.5
Hibiscus rosa-sinensis	Flower	1	1	1	1	1	1
Sapindus mukorossi	Fruit	3	3	3	3	3	3
Acacia concinna	Fruit	3	3	3	3	3	3
Glycyrrhiza glabra	Root	1.5	1.5	1.5	1.5	1.5	1.5
Citrus sinensis	Peel	1.5	1.5	1.5	1.5	1.5	1.5
Triethanolamine lauryl sulfate	–	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL
Methylparaben	–	0.25	0.25	0.25	0.25	0.25	0.25
Water	–	Q.S.	–	–	–	–	–
Perfume		Q.S.	–	–	–	–	–

containing 50 mL of the same shampoo solution. Height of the column is measured at specified times, for example, height can be measured immediately after the shampoo solution has passed through the apparatus and 5 min thereafter. The height of column considered to be proportional to volume. The effect of hard water can also be studied by this method.

Detergency and cleaning action

Barnet and powers test place 5 g of wool yarn in grease in 200 mL of water containing 1 g of shampoo in flask. Shake the flask, 4 min (rate, 50 times in a minute), temperature of water was 35°C, after removing the solution, the sample was takeout. Dry the sample and weigh it. The amount of removed soil was calculated.

Wetting action

The Draves–Clarkson test is a standard method to determine the effectiveness of wetting of cotton skeins. This test was originally developed for evaluating products meant assisting in dyeing cotton yarns and fabrics. Although there is no relation between dyeing of cotton yarn or fabric and shampooing, this test can be used to study wetting action.[11]

Table 2: Evaluation of formulation for physical appearance and pH

Formulation	Physical appearance	pH
F1	Dark brown	5.23
F2	Dark brown	5.42
F3	Dark brown	5.22
F4	Dark brown	5.62
F5	Dark brown	5.51
F6	Dark brown	5.42

Table 3: Viscosities and solid contents of developed formulations

Formulation	Viscosity (Cp, at 10 rpm)	% solid contents
F1	1260	21.02
F2	2478	23.05
F3	4259	24.10
F4	5789	27.02
F5	6259	28.06
F6	9058	29.01

Surface tension measurement

At room temperature, 10% of shampoo dilution in distilled water was measured and thoroughly clean the Stalagmometer by water and acetic acid.

The data were calculated by the following equation:

R3 = (W3-W1) n1 X R1, (W2-W1) n2

Where,
W1= Weight of empty beaker.
W2= Weight of beaker of distilled water.
W3= Weight of beaker of shampoo solution.

Stability studies

The antifungal activity of the formulations was compared for samples stored at room temperature (25°C and 45°C) and in the refrigerator (4°C) as well as those packaged into glass containers versus plastic containers. In addition to the antibacterial activity, pH values, color, physical appearance, and texture were also tested during the 3 months with the above-described methods.[12]

Screening of antifungal activity

Preparation of media

Ingredients (Gms/Liter)

- Part A - Malt extract 36.000 peptone 36.000 Ox-bile desiccated 20.000 Agar 14.500.
- Part B - Tween 40 10.000 Glycerol monooleate 5.000.
- Final pH (at 25°C) 6.0±0.2.
- Formula adjusted, standardized to suit performance parameters.

Directions - Suspend 15 mL of fluid Part B in 1000 mL distilled/purified water. Add 106.5 g of Part A. Mix well and heat to boiling to dissolve the medium completely. Sterilized by autoclave at 15 Pounds pressure (121°C), time of rest is

Table 4: Cleansing, surface tension, and detergency parameters of developed formulation

Formulations	Cleaning (%)	Surface tension (dynes/cm)	Detergency (%)
F1	25.50	28.24	58.53
F2	27.21	29.05	60.22
F3	27.52	31.21	61.28
F4	28.16	32.42	62.24
F5	29.23	33.20	62.88
F6	29.72	33.55	63.21

15 min. Cool to 45–50°C. Mix well and pour into sterile Petri plates or dispense into tubes for slants.[13]

Subculture preparation

A day before testing done, inoculation of the culture was made in Dixon agar media at 37°C for 18–24 h.

Test solution preparation

5 g of test compound were dissolved in 5 mL distilled water to give 1000 g per milliliter (this solution was used for testing).

Table 5: Evaluation of foam stability of polyherbal shampoo formulations

Time (min)	Foam volume					
	F1	F2	F3	F4	F5	F6
1 min	173	170	172	175	171	172
2 min	172	169	170	173	169	171
3 min	170	167	169	172	167	168
4 min	169	166	167	170	166	167
5 min	167	165	166	169	164	166

Antimicrobial activity (testing method)

7 mm diameter Whatman filter paper disc was prepared, dipped, and sterilized in test and ketoconazole (standard drug), each disc had absorbed approximately 0.08 mL of solution. Both reference and standard, i.e., ketoconazole discs were placed aseptically at opposite directions of each other over nutrient agar plates seeded with respective test microorganism (with ethanol dipped flamed forceps).

Incubation

Plates were placed upright position at 37°C for 24–48 h. Diameter of incubation zones (in mm) and inhibition of zone (in %) was calculated.[14]

RESULTS

A pure herbal shampoo was formulated by mixing aqueous extracts of pomegranate, reetha, shikakai, orange peel, licorice, curry leaves, neem leaves, and hibiscus flower definite amount as shown in Table 1. These plant materials contain phytochemicals such as saponins which are natural surfactants possessing good detergency, conditioning and foaming properties.A good shampoo must have adequate viscosity to facilitate removal

Table 6: Stability studies of optimized formulation (F6)

Parameters	After 1 month	After 2 months	After 3 months
Physical appearance/visual inspection	Clear	Clear	Clear
pH	5.20	5.23	5.26
Solids contents (%)	24.13	23.78	23.56
Surface tension measurement (dy/cm)	31.69	32.21	32.43
Viscosity	9057	7043	6352
Detergency ability (%)	66.21	64.23	62.77
Foaming ability and foam stability (mL)	170	168	166

Table 7: Antimicrobial activity of polyherbal shampoo (50 mL)

Formulation	Test organism	Concentrations (mg/mL) (zone of inhibition in mm)			
		50	100	150	200
F1	*M. furfur*	21	20	18	16
F2	*M. furfur*	22	20	18	17
F3	*M. furfur*	23	18	16	15
F4	*M. furfur*	24	20	17	16
F5	*M. furfur*	25	23	21	19
F6	*M. furfur*	28	26	24	21
Base	*M. furfur*	10	8	7	5
Synthetic marketed product	*M. furfur*	30	28	27	25
Herbal marketed product	*M. furfur*	28	27	26	25

M. furfur: *Malassezia furfur*

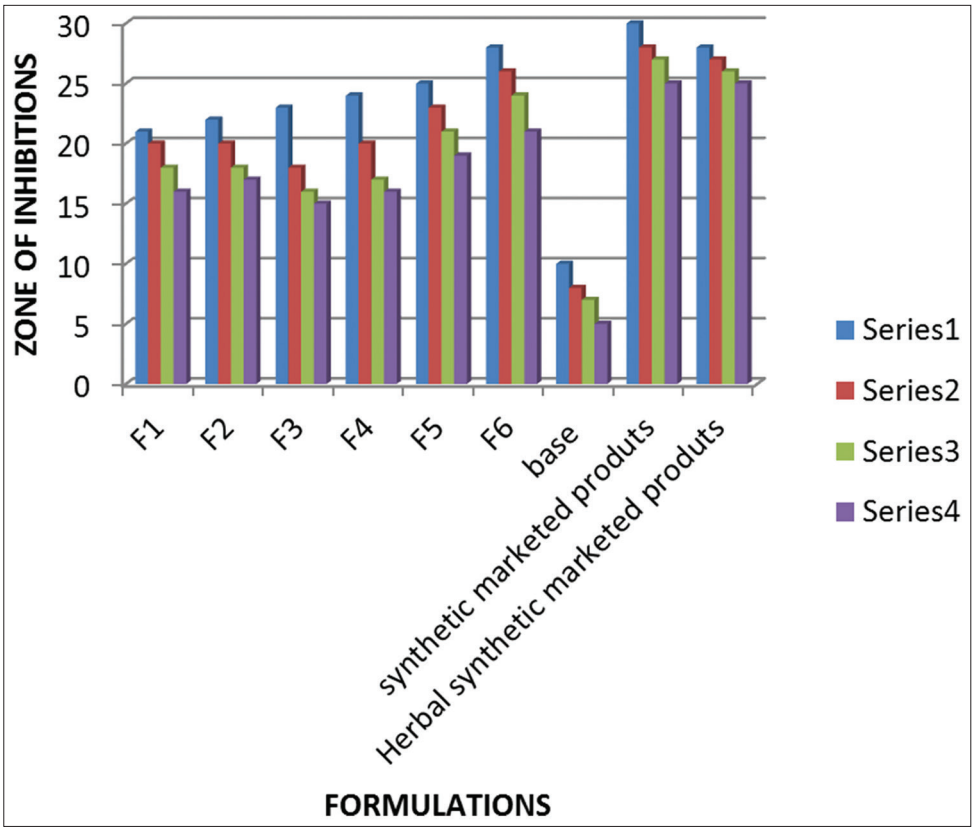

Figure 1: Antifungal activities of prepared formulations [F1-F6]

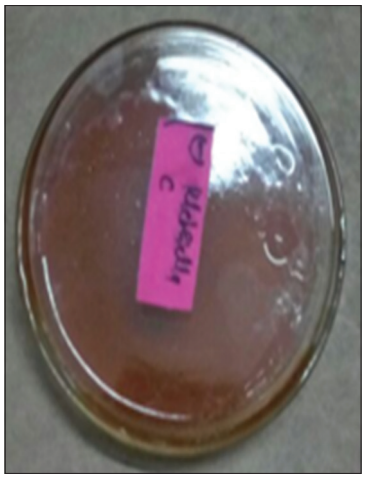

Figure 2: Nutrient media plate

from the bottle but must not drip down from the hair during use. A variety of natural materials is available for use as viscosity builders. Shampoo was further preserved by the addition of little amount of methylparaben. The best result was obtain from formulation F6 in terms of all parameters.

DISCUSSION

The pH of shampoos has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes, and stabilizing the ecological balance of the scalp. All six formulations in Table 2 shows that the shampoo were acid balanced (5.2-5.6) which I near to skin pH.

The result of viscosity and percent of solid's contents is tabulated in Table 3, and percent of solids was found between 21.02% and 29.01%. As a result, they were easy to wash out. If the shampoo has too many solids, it will be hard to work into the hair or too hard to wash out viscosity of all six formulations increased with increase in concentration. F1 showing lesser viscosity when compared to formulation and F6 shows higher. To better observe the difference between shampoos, the viscosity at a low rotational speed (10 rpm) was compared. As we found from the literature the share rate are about 5-10 rpm.

According to mentioned in literature that a shampoo formulation should be able to down the surface tension of water to about 39 dynes/cm. surface tension of water reduced to 34.2 dynes/cm from 72.8 dynes/cm by herbal shampoo, i.e., a clear indication of shampoos detergency as show in Table 4. Cleaning was done on wool yam and grease. As the results seem, significant difference in the sebum removed amount by the different shampoo. F6 shows good cleaning property. All the six shampoo preparations showed similar foaming characteristics in distilled water, comparable foaming properties, foam stability of herbal shampoos is listed in Table 5.

Durinthe long time storage, acceptability and stability of physical properties (color and odor) of formulations indicate

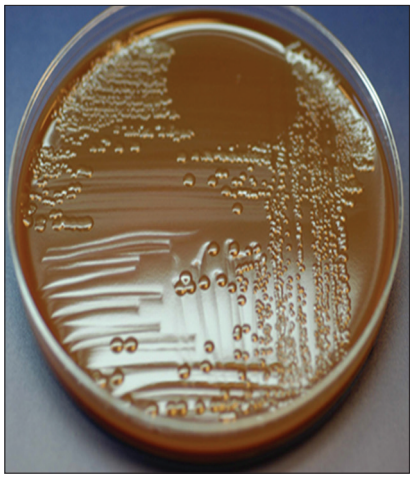

Figure 3: Plate after inoculation

Figure 4: Plate after fungus growth

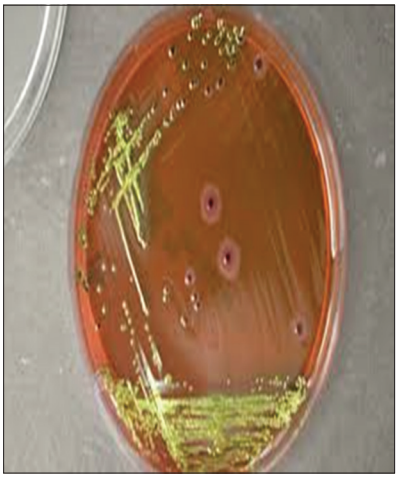

Figure 5: Zone of inhibition F1 and F2

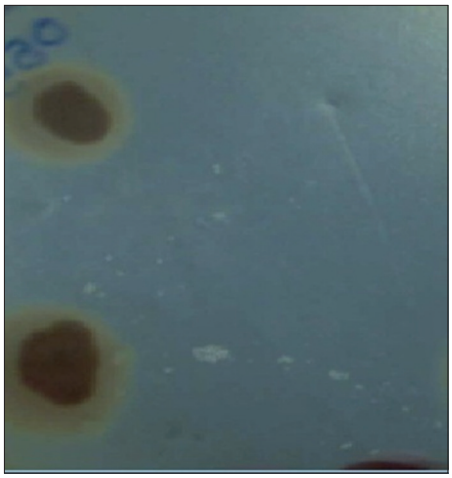

Figure 6: Zone of inhibition F3 and F4

Figure 7: Zone of inhibition F5 and F6

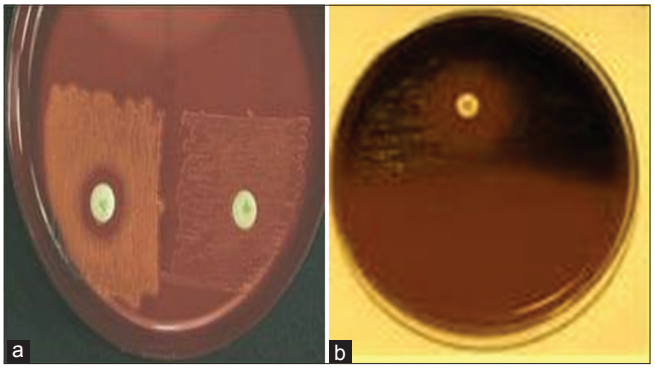

Figure 8: (a and b) Zone of inhibition of marketed formulations

that they are physically and chemically stable. Table 6 shows the stability of F6 in terms of all parameters is not changed as compare it to the previous formulations. The results of antifungal activity are described and Table 7 shows the result of antifungal activity of all formulation is describe in Figure 1 zone of inhibition of F1, F2, F3, F4, F5, and F6 was shown in Figures 2-8, respectively. The formulation F6 showed maximum zone of inhibition. Therefore, it is concluded that as the concentration of the herbs increased, the zone of inhibition was also increased; hence, the formulation F6 was the best formulation for treating dandruff. The same was compared with marketed product, synthetic and herbal anti-dandruff shampoo containing ketoconazole (Nuforce shampoo) as an active ingredient and Kesh kanti (Patanjali) used as herbal anti-dandruff shampoo. The result found that

the formulated product shows better control over dandruff near the marketed product.

CONCLUSION

The aim of this study was to formulate a completely herbal shampoo which is at par with the synthetic shampoo available in the market. We formulated a polyherbal shampoo using plant extract which is commonly used traditionally and lauded for their hair cleansing action across Asia. All the ingredients used to formulate shampoo are safer than silicones and polyquaterniums synthetic conditioning agents. Instead of using cationic conditioners, we have used shikakai, hibiscus reetha, and other plant extracts to provide 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 (F6) containing different concentrations of herbal extract of pomegranate, reetha, shikakai, orange peel, licorice, curry leaves, neem leaves, and hibiscus flower could be used as an effective in the treatment of dandruff on scalp. Pomegranate is the new and key ingredient for *M. furfir* species as antifungal. It shows good antifungal effect in combination with increased concentration. Several tests were performed for physicochemical properties of prepared shampoo for quality control test, but further research and development are required to improve its overall quality.

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