Formulation Development and Evaluation of Herbal Antidandruff Shampoo.

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Abstract
Synthetic detergents and preservatives sometimes been cause of adverse effect among consumers. Basically, Alkanolamides are used for the formation of stable foam; but because of producing nitrosamines, they are potentially carcinogenic compounds. A more radical approach in reducing the synthetic ingredients is by incorporating natural extracts whose functionally is comparable with their synthetic ingredients. Hence, an important task is to develop completely natural shampoo without carcinogenic compounds. Materials and methods: To formulation of herbal shampoo by using three different bases. In the present investigation, physico-chemical parameters, conditioning effect, ophthalmic irritancy (CAM-TBS assay), antifungal potential against Malassezia furfur, Candida albicans, Trichophyton rubrum, Microsporum gypseum and stability study comparison with commercial herbal shampoo were evaluated. Completely natural shampoos were formulated in the laboratory and their conditioning effects were evaluated by scanning electron microscope study. Results and Discussion: The laboratory formulation (Formulation C: pearl surfactant natural base, New Zealand) were found to be better with respect to hair damage than the formulation B which was formulated with Sodium lauryl ether sulphate base. Formulation C provided stable foam, surface tension reduction, good cleaning and wetting effect. Moreover, the aesthetic attributes, such as lather and clarity were comparable with the marketed formulation. High performance thin layer chromatography (HPTLC) fingerprinting was also performed to confirm chemical characteristic of the formulation, due this phytoconstituents resulted strong antifungal effect. Conclusion: In every aspect, formulated herbal antidandruff shampoo with Pearl surfactant natural base was found to be better than standard.

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Keywords: Antidandruff shampoo, stability, CAM, HPTLC, Conditioning effect, Antifungal activity.

1. Introduction
Natural cosmetics are popular one all over the world as they convey the impression of having better purity, safety and efficacy. Many shampoos are available in Indian market under ‘natural’ or ‘herbal’, etc. however; these formulations contain synthetic detergents and other chemical additives. Our laboratory is presently involved in the development of a completely natural shampoo, in which all the ingredients are either plant derived material. The general feeling among consumers is that a good shampoo is one that foams very
well. However, formulating a shampoo using all natural raw materials is a formidable task. The challenge lies not only in selecting materials that can be rationally justified as ‘natural’, but also matching the performance characteristics of the formulation with synthetic ones available in the market[1]. The increasing variety and large number of chemicals introduced onto the market and also into the environment each year, and the resulting requirements for protection of human health and the human environment, have necessitated the monitoring of environmental materials and specimen banking as well as the development of rapid and reliable methods for toxicity evaluation and risk assessment[2]. The damage provoked by some substances on the chicken egg’s chorioallantoic membrane (CAM) with trypan blue staining is used as an alternative assay to determine ocular irritation [3]. In present study, formulate herbal antidendruff shampoo using natural extracts and evaluated Physico-chemical parameters, conditioning effects, antifungal activity and the eye irritation potential of formulation a using the CAM-TBS in vitro method, this determination is very simple, reproducible and quantitative. The CAM-TBS assay uses trypan blue absorption as indicator of chorioallantoic membrane injury and showed a good correlation with the in vivo Draize eye irritation test in an evaluation of substances.

2. Material and methods:

2.1 Preparation of crude extracts: Brassica nigra, cassia fistula, Cassia tora, Glycyrrhiza glabra, Saussarea lappa, Bucchania lanzan, Emblica officinalis which antimicrobial and conioning properties already reported were homogenized and extracted using ethanol (70% v/v). The extract were filtered and concentrated to dryness under reduced pressure and controlled temperature (50-55°C) to obtain solvent free semisolid extracts. The solvent free extracts was washed, weighed and packed into plastic containers and stored in room temperature.

2.2 Formulations Development of herbal antidendruff shampoo

Formulation of three different clear liquid antidendruff shampoo using different bases, such as saponin (Sapindus mukorossi, ritha) as natural surfactant, Sodium laurel ether sulphate (SLES), Pearle surfactant natural base (Cocamidopropyl betaine, Cocamide Diethanolamines, Glycol distearate) obtained from Aromatics and More Ltd., Newzeland. Apart from surfactant some additives were added in shampoo formulation. Additives play an important role in defining the performance, stability, and aesthetic appeal of any cosmetic formulation. A variety of natural materials available for use as viscosity builder. Here we have use Hibiscus juice, Xanthane gum, Carbopol, PEG as viscosity modifier, citric acid as chelating agent and sodium chloride as a thickener in the formulation. The antifungal herbs such as B.lanzan, B.nigra, C.tora, C.fistula, E.officinalis, G.glabra, S.lappa, and V.mungo were chosen for formulation development of shampoo [1, 4].

2.3 Physico-chemical evaluation of shampoo

2.3.1 P$^H$

The P$^H$ of the 10% shampoo formulation in distilled water was determined at 25°C by Digital P$^H$ meter [5].

2.3.2 Total Solids

Weigh accurately quantity of shampoo under examination placed in tared dish. Approximately 4 gm of shampoo added to the evaporating dish, shampoo was evaporated at as low temperature as possible until the solvent was removed. Calculate & record the weight of shampoo solids after drying [6].

2.3.3 Viscosity

The viscosity profile of the shampoo formulation was measured using Brookfield viscometer DV-E model at 25° C [7]. (Brookfield engineering labs, USA)

2.3.4 Dirt dispersion test

Put two drops of shampoo formulation in a large test tube. Add 10 ml of distilled water and 1 drop of Indian ink. Stopper the test tube and shake it for ten times. Estimate the amount of ink in the foam as None, Light, Moderate, or Heavy and record. Shampoos that cause the ink to concentrate in the foam are considered as poor quality. The dirt should stay in the water portion. Dirt that stays in the foam will be difficult to rinse away. It will redeposit on the hair [5].

2.3.5 Foam formation

Shake test: Approx. 50 ml of the 1% shampoo solution was put into a 100 ml graduated cylinder and shake for10 times and the volume were recorded [5].

2.3.6 Foam retention

Immediately after the Shake test, reduction volume of foam at 1-minute intervals for 4 minutes was recorded [5].

2.3.7 Wetting time

Wetting time was measured by Drave’s test, when weighed skein of cotton was allowed to sink through a wetting solution in a 500 ml graduated cylinder, the time taken for sinking was given as wetting efficiency. The lower time required for sinking, higher is the wetting efficiency. This method is alternative to canvas disc method; it is more accurate, time saving method [7].

2.3.8 Detergency Evaluation

Although cleaning or soil/Sebum removal is the primary aim of shampoo, experimental detergency evaluation has been difficult to standardize, as there is no real agreement on a standard soil, a reproducible soiling process or the amount of soil a shampoo should ideally remove.

Hair tresses of Asian (Indian) origin where obtained from local market, the tresses were prewashed with 7% SLS solution, dried and cut into 6 inch, 3 gm swatches. The sebum composition was choosen to include a variety of functional groups similar to that in actual sebum. The hair swatch (3gms) was suspended in 20 ml of a 10% sebum solution (olive oil25%, coconut oil10%, stearic acid 15%, oleic acid 15%, paraffin wax 15%, and cholesterol 25%) in hexane for 15 min with intermittent shaking. The swatch was
removed, the solvent evaporated at room temperature and hair swatch weighed to determine the sebum load. Three swatches were treated similarly, each swatch was then split into two equal samples of 1.5 gm each, one for shampoo treatment and other to act as an internal control. The test swatch was washed with 0.1 ml of 10% shampoo solution using finger method. It was then dried using a hair dryer and further dried in oven at 60°C for 4 hours to ensure uniform moisture content.

The sebum remaining in the test swatch after shampooing and unwashed control swatches was then extracted, using 20 ml hexane in a stopper flask for 40 min. on a rotary shaker. The hexane solution was then evaporated to dryness and sebum extracted from the test and control swatch was weighed [7].

The detergency was evaluated as percentage of sebum removed after shampooing:

\[ \text{Percentage detergency} = \frac{\text{Weight of sebum in test swatch C}}{\text{Weight of sebum in control swatch}} \times 100 \]

Where, T: Weight of sebum in test swatch C: Weight of sebum in control swatch.

2.3.9 Conditioning effect of formulation with scanning electron microscope (SEM)
The scanning electron microscope (SEM) is a valuable tool to demonstrate the effects of various toiletry treatments on hair. Conditioning effect evaluated by scanning electron microscope. The scanning electron microscope (SEM) was used to determine the conditioning effects shampoo formulation. The hair strands were immersed in 20ml of the shampoo solution and agitated mildly on a rotary shaker for 10min. The solution was siphoned off and the hair strands were subjected to rinsing for a period of 1min with 20ml of distilled water and repeated once. Finally, the hair was dried by gently pressing between pieces of filter paper and was stored at 75%RH.

The hair strand were mounted under SEM, The micrographs were taken at three different magnifications 500 X, 1500 X and 3000 X. At 1500X magnification, the scale surfaces and their edges became clearer and any scale uplift could be identified [8].

2.4 Determination of in vitro ocular irritancy by Chorioallantoic membrane test with trypan blue staining

The test was carried out as per methods published under Protocol Number 108 of INVITOX (11). Fertile White hen’s eggs, at 12 days of incubation, weighing between 55 g-65 g, obtained from Government animal production laboratory Nashik, were used in the assays. Six eggs were used for each test sample. On 10th day of incubation, the eggs shell above the air space was removed. The exposed membrane was moistened with a drop of 0.9% physiological saline and the saline was washed to exposing the CAM. An aliquot of 200 µL of the test substance was applied onto the CAM for 30s and washed with enough distilled water to remove test sample. Next, the CAM was treated with 500 µL of 0.1% trypan blue solution for 2 min. Excess of dye was rinsed off with distilled water. The dyed CAM was excised and extracted with 5 mL formamide, and absorbance of the extract was measured spectrophotometrically at 595 nm. The absorbed trypan blue was determined from a calibration curve by triplicate (10⁻⁶ M, 10⁻⁵ M, 5 × 10⁻⁷ M) of trypan blue in formamide [9, 10].

2.5 HPTLC fingerprinting of Shampoo formulation [12]

HPTLC is ideally suited for fingerprinting because it is very rapid, reliable, easy to use and maintain, provides a lot of instrumental and visual data. High Performance Thin Layer Chromatographic (HPTLC) fingerprint data, comparison of a “standard” with that of a sample can be accepted as the rapid, reliable and modern procedure for routine quality control.

**Instrumentation and chromatographic conditions:**
The samples were spotted in the form of bands of width 6mm with a Camag microliter syringe on precoated silica gel aluminium plate 60F-254(10 x 10 cm) with 200µm thickness (E. merck, Germany) using a Camag Linomat V (Switzerland) sample applicator. A constant application rate 10 microliter was employed & space between two bands was 10mm. Linear ascending development was carried out in a twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 20 min. at room temperature. The length of chromatogram run was 80 mm. subsequent to the development. The TLC plates were dried and densitometric scanning was performed on Camag TLC scanner III at 354, 366, 280, & 364 nm for monoaminoglycyrrizhinate (MAG), gallic acid, Sennoside, β-sitosterol respectively.

**Analysis of Monoaminoglycyrrizhinate, gallic acid, Sennoside, β-sitosterol:**

An accurately weighed 5ml shampoo formulation was extracted with 25 ml methanol by sonication for 30 min. This extract was centrifuged at 12,000 rpm for 15 min. at 4°C. The supernatant was filtered, dried at room temperature. The residue was redissolved in 5ml methanol. An accurately weighed 1mg Standard sample of Monoaminoglycyrrizhinate, gallic acid, Sennoside was dissolved in methanol and β-sitosterol in chloroform. A 10 µl of the filtered solution was applied on the TLC plate followed development in different mobile phases, Chloroform: Methanol: Glacial acetic acid (3:6:0.6:1.6:0.2:0.2v/v) for Monoaminoglycyrrizhinate, Ethyl acetate: Toluene: Ethyl acetate: Water: Formic acid (25:25:50:50:50v/v) for Sennoside, Chloroform: Methanol (10:1) for β-sitosterol. After the development HPTLC plate was derivatives with spraying reagent and scanning.

2.6 Antifungal activity

2.6.1 By direct pouring method

Sample preparation: Each shampoo in the study was used in its concentrated form and in 10-fold serial dilutions using sterile distilled water up to a dilution of 10⁻⁵ Sabouraud’s dextrose agar (1.5% agar) with 1% olive oil added to the medium was used throughout the study. The oil was mixed prior to autoclaving at 15 psi for 15 min. The autoclaved medium was allowed to cool and then sufficient medium was
transfer into Petri dishes. Two ml of the each shampoo dilutions and one ml of the each organism suspension were also added into the Petri dishes and gently rocked to disperse all three ingredients. All plates were incubated at 37 °C. After five days incubation at 37 °C, all plates were read for growth [13].

Table I. Formulation of Herbal Antidandruff Shampoos with using different bases

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation A</th>
<th>Formulation B</th>
<th>Formulation C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant</td>
<td>Saponin (25%)</td>
<td>SLES (33%)</td>
<td>Pearl (35%)</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Viscosity builders</td>
<td>Hibiscus juice (15%)</td>
<td>PEG (3%), Carbopol (3%), Xanthane gum (3%)</td>
<td></td>
</tr>
<tr>
<td>Honey</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Herbal extract</td>
<td>12%</td>
<td>12%</td>
<td>12%</td>
</tr>
<tr>
<td>Essential oil</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q. s.</td>
<td>q. s.</td>
<td>q. s.</td>
</tr>
</tbody>
</table>

Table II: Physico-chemical properties of formulated shampoo formulation

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Test parameter</th>
<th>Laboratory Shampoo formulation</th>
<th>Commercial shampoo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>7.466±0.017</td>
<td>7.31±0.014</td>
</tr>
<tr>
<td>2</td>
<td>Total solids (%)</td>
<td>29.83±0.600</td>
<td>32.16±0.722</td>
</tr>
<tr>
<td>3</td>
<td>Viscosity (c. p.)</td>
<td>48.00±0.000</td>
<td>46.00±0.000</td>
</tr>
<tr>
<td>4</td>
<td>Foam formation(mL)</td>
<td>54.66±0.333</td>
<td>81.33±0.666</td>
</tr>
<tr>
<td>5</td>
<td>Foam retention(mL)</td>
<td>52.33±0.333</td>
<td>79.33±0.666</td>
</tr>
<tr>
<td>6</td>
<td>Wetting time(s.)</td>
<td>14.66±0.888</td>
<td>11.66±0.888</td>
</tr>
<tr>
<td>7</td>
<td>Detergency evaluation (%)</td>
<td>81.78±0.971</td>
<td>78.89±0.455</td>
</tr>
<tr>
<td>8</td>
<td>Dirt dispersion</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Table III. In vitro irritancy of Laboratory shampoo formulation by CAM-TBS test

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Absorbance of extracted CAM at 595 nm.</th>
<th>Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.905</td>
<td>4.363</td>
</tr>
<tr>
<td>B</td>
<td>3.082</td>
<td>3.167</td>
</tr>
<tr>
<td>C</td>
<td>3.877</td>
<td>3.606</td>
</tr>
</tbody>
</table>

Table IV: In vivo Irritancy classification of laboratory shampoo formulation

<table>
<thead>
<tr>
<th>Test substances</th>
<th>In vivo classification</th>
<th>Trypan blue absorbed (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampoo A</td>
<td>NI</td>
<td>1.92 ± 0.17</td>
</tr>
<tr>
<td>Shampoo B</td>
<td>NI</td>
<td>1.24 ± 0.06</td>
</tr>
<tr>
<td>Shampoo C</td>
<td>NI</td>
<td>1.67 ± 0.10</td>
</tr>
</tbody>
</table>

NI: non irritant

Table V. Antifungal effect of different shampoo formulations and standard (Vatika antidandruff shampoo) on different test organisms.

<table>
<thead>
<tr>
<th>Formulation (Conc.)</th>
<th>M. furfur</th>
<th>C. albicans</th>
<th>T. gypseum</th>
<th>M. rubrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>A 100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A 1000</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>B 10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B 100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B 1000</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>C 10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>C 100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C 1000</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Std.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

--: clear (completely inhibition), +: dense (partially inhibition), ++: highly dense (no inhibition).
2.6.2 By Zone of inhibition study
Formulation A, B, C, and M were used for the study. Each shampoo in the study was used in its concentrated form and in two-fold serial dilutions using sterile distilled water up to a dilution of \(10^{-7}\). Sabouraud’s dextrose agar (1.5% agar) with 1% olive oil added to the medium was used throughout the study. The oil was mixed prior to autoclaving at 15 psi for 15 min. The inoculums of test organisms were spreaded on SDA plates. Wells of 6 mm were punched into the agar medium and filled with 0.1 ml of shampoo dilutions. The plates were incubated for 24 hours at 37°C. The antifungal activity was evaluated by measuring the zone of inhibition and compared with control. Diameter of zone of inhibition measured by digital vernier caliper [14].

2.7 Accelerated stability Study
The shampoo formulation (A, B, C) were packed in 3 bottles each containing 100 ml. These bottles were properly labelled. They were kept in 3 different environmental conditions, i.e. 3 different stability chambers with 3 different storage conditions. The stability conditions were adjusted as per ICH guidelines i.e. taking care about temperature and relative humidity 40°C ± 2°C/ 75%RH, 35°C ± 2°C/ 65 % RH and at room temperature. i.e. 30°C ± 2°C/60 % RH [15, 16].

3. Results:
The table II showed that the \(pH\) of standard (M) and in house formulation (A, B, and C) was 7.46, 7.31, 7.75 and 8.32 respectively within the limit prescribe. Total solids content in the shampoo should not be exceeding than 40 %. Total solids of formulation C 22.66±0.60 was less than standard shampoo formulation 29.50 ± 0.28.

Product rheology plays an important role in defining and controlling many attributes such as shelf life, stability and product aesthetics value such as clarity, pourability, spreading capacity on hair and product consistency in the package. Therefore viscosity evaluation is of paramount impotence in the study. Table no. II indicates that Shampoo A & B are low viscosity products than C (284 c. p.). Although foam generation has little to do with the cleansing ability of shampoos, it is of paramount importance to consumer and is there of an important criterion in evaluating shampoos. The total Foam volume was found to be 54.66 ± 0.33, 81.33 ± 0.66, 83.00 ± 0.57 and 82.33 ± 0.33 ml for in house and standard formulation and it shows foam drainage time up to 2 ml. Foam drainage represent the life of foam which is of paramount importance to consumer and is there of an important criterion in evaluating shampoos. The rate of wetting or wetting ability of surface-active agent is commonly used to determine their comparative efficacies. The wetting ability of substance is functioning of its concentration.

In Fig.1, the scale edges are highly indented and non-uniform and some degree of scale uplift are also visible. The upper surface of the hair shaft in Fig. 2 shows gross cuticle damage due to SLES. The scale edges have eroded to a major extent and are highly irregular. The lack of conditioning is evident in this case while in Fig.3, the scale edges are smoothly curving and uniform and it appear tightly flattened on the underlying cells, indicating very good conditioning effects of shampoo formulation C. In commercial shampoo (Fig.4) highly jagged scale edges are observed but the scales are seen to lie on flat one over the other.

![Figure 1](image)

**Figure 1** SEM photograph shows conditioning effect of shampoo on hair sample after treated with formulation A

Calibration curve for trypan blue showed a high correlation of \(r = 0.9473(p < 0.01)\) Table III And Graph I. It was used to quantify the amount of trypan blue absorbed by CAM for all shampoo formulation. The results are presented Table II as the mean ± standard deviation of the amount of trypan blue absorbed following incubation with the test substance. Table IV Showed the eye irritation score of shampoo formulation obtained in the in vitro assay can be classified according to the amount of trypan blue absorbed (nmol) as non-irritant/weak irritant <7.0, moderate irritant 7.0–14.5 and severe irritant >14.5. These values were determined using the regression formula \(Y = 1.454x + 1.378\). In-house shampoo formulation a CAM was absorbed less dye it may be due to addition of plant extracts.

**HPTLC chromatogram** Figure 5-8 represent \(R_f\) value of Monoaminoglycyrrizhinate (0.91), Gallic acid (0.62), Sennoside (0.44), \(\beta\)-sitosterol (0.84) in shampoo formulation C was nearly same as compared to standard 0.92, 0.62, 0.43, 0.83 respectively. According to HPTLC fingerprinting & \(R_f\) value of reference compound, biomarker was present in the
Figure 2. SEM photograph shows conditioning effect of hair sample after treated with formulation B.

Figure 3. SEM photographs shows conditioning effect of hair sample after treated with formulation C.

Figure 4. SEM photographs shows conditioning effect of hair sample after treated with formulation M.

Figure 5. HPTLC fingerprint of monoaminoglycyrrhizinate (biomarker) in shampoo formulation.
formulation due to addition of herbal extract of drugs like Glycyrrhiza glabra, Embelica officinalis, Cassia tora and Cassia fistula which having antimicrobial potential to reduce microbial load on scalp and retaining conditioning effect.

Figure 6 HPTLC fingerprint of gallic acid
In the direct pouring method shampoo formulations were judged better in killing action on the test organisms even at a dilute concentration (1:10) also partial killing occurred in the shampoo at very dilute (1:100) concentration. The test shampoo was 10 times more effective than the marketed antidandruff shampoo (Vatika). The shampoo formulation exhibited clear plate at the conc. 1:10, slightly dense at conc. 1:100 and denser at the conc. 1:1000 (Table V). When growth was present, & the plates were opaque (dense) with the presence of the yeast, this was interpreted to mean that no antifungal activity had occurred at that concentration of the test shampoo. When plates were appeared to be clear, this was interpreted to mean that the yeast had been killed by that concentration of the test shampoo. All prepared herbal shampoo formulations have better antifungal action as compared to standard.

In the zone of inhibition study a result was expressed as the reciprocal of the highest dilution at which no fungal growth occurred. The shampoo formulations were diluted up to 300 times by preparing two fold dilutions. The shampoo formulations exerted a greater inhibitory activity at 1: 256 (Table VI and graph 2).

The accelerated stability study of in house herbal antidandruff shampoo formulation were summarized in Table V and there was no significant change observed in the value of physico-chemical parameter,
Table VI. Concentration of shampoo formulation for inhibition of fungal growth

<table>
<thead>
<tr>
<th>Formulation</th>
<th>M. furfur</th>
<th>C. albicans</th>
<th>T. rubrum</th>
<th>M. gypseum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1: 256</td>
<td>1:256</td>
<td>1: 64</td>
<td>1: 64</td>
</tr>
<tr>
<td>B</td>
<td>1: 256</td>
<td>1:256</td>
<td>1: 64</td>
<td>1: 64</td>
</tr>
<tr>
<td>C</td>
<td>1: 256</td>
<td>1:256</td>
<td>1: 64</td>
<td>1: 64</td>
</tr>
</tbody>
</table>

A: saponin shampoo,   B: SLES shampoo,   C: pearl shampoo

Graph 1: Linearity of trypan blue

Graph 2: Antifungal activity (zone of inhibition) of shampoo formulation by agar well plate method.

4. Discussion:
The pH of the shampoos has been shown to be important for improving and enhancing the qualities of hair, minimizing the irritation to the eyes and stabilizing the ecological balance of the scalp. The current trend to promote shampoos of lower pH is one of the ways to minimize damage to the hair. Mild acidity prevents swelling and promotes tightening of the scales, thereby inducing sheen. Detergency of in-house formulation C was shown similar to standard marketed shampoo. Cleansing and soil/sebum removal action is the primary aim of shampoo. However, it should not have more detergency because it removes natural oil from hair and gives the drying-out effect. Results obtained from this study indicate that detergency of in house and commercial formulation within the range of 78-85%. In comparative studies of conditioning effect of different test shampoos, formulation C gives very good conditioning effect on hair. This indicates that despite the damage to the cuticle by the detergent, the conditioning agents used in the shampoo formulation are successful in preventing cuticle uplift. From the CAM-TBS assay we conclude that prepared in-house shampoo formulations were non-irritant, it doesn’t produce irritation to the eye. HPTLC is a new approach which may lead to proper standardization of shampoo formulation based on fingerprinting characteristics. Hence we could conclude that it is a very crucial step for quality control of herbal Shampoo. Results obtained from these studies indicated that all the herbal shampoo formulations exhibited significant inhibitory action on \( C. \text{ albicans}, \ M. \text{ furfur}, \ T. \text{ rubrum} \) and \( M. \text{ gypseum} \) and could act as a good antifungal and antidiandruff formulation. It appears from the study that there were considerable differences in antifungal activity between these shampoo formulations. Amongst all the formulation, formulation ‘C’ has the maximum inhibitory effect against \( C. \text{ albicans}, \ M. \text{ furfur}, \ T. \text{ rubrum} \) and \( M. \text{ gypseum} \) and it was designated as best formulation.

5. Conclusion:
From the present study, tested shampoo formulations possess all evaluation parameters which satisfy an ideal shampoo property. Furthermore they were prepared in herbal base which had its own advantages over synthetic. Hence we could conclude that it is a very crucial step for quality control of herbal Shampoo. All above parameters were done on the standard Marketed preparation and in-house herbal antidiandruff shampoo and it showed the entire test results within range. Nowadays there is strong demand for natural therapies, and this is increasing in western countries. The
herbs which are a cheapest of phytoconstituents are on wheals to attain their role in Polyherbal formulation so as to have synergistic role. Hence we conclude that the Polyherbal Formulation of Shampoo is effective in reducing dandruff without irritation, less adverse effect and better conditioning effect. In the present scenario, it seems improbable that herbal shampoo, although better in performance and safer than the synthetic ones, will be popular with the consumers. Amore radical approach in popularizing herbal shampoo would be to change the consumer expectations from a shampoo, with emphasis on safety and efficacy. Formulators must play an active role in educating the consumers about the potential harmful effects of synthetic detergents and other chemical additives present in shampoos. There is a strong need to change the consumer perception of a good shampoo and the onus lies with the formulators.

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7. References:

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