

IN-VITRO ANTI-DANDRUFF ACTIVITY OF HERBAL SHAMPOO FORMULATION-A RESEARCH ARTICLE

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Article Received date: 21 December 2024

Article Revised date: 15 January 2024

Article Accepted date: 06 Feb. 2024



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ABSTRACT

The objective of the present research work is to formulate herbal shampoo using traditional herbs like Castor, Henna, Reetha, Neem Tulsi as a replacement to synthetic surfactants and carryout a comparative analysis using marketed products. Herbal shampoos formulation was carried out using peppermint, Lemon & Hibiscus extract powders at different concentrations. Formulated shampoos evaluated for organoleptic evaluation, powder characteristics, pH, dirt dispersion, detergency, cleaning action, foaming ability and its stability and conditioning performance. Design Expert Statistical software used to optimize the formulations and to understand main effects, interaction effects on the properties of shampoos. All the herbal powder shampoo formulations pH closer to the skin pH. The shampoos containing higher concentration of Reetha & Tulsi shown higher detergency, Henna, Castor as well as peppermint showed significant effect on the foaming capacity and cleaning action and similar to marketed formulations. Higher quantity of Reetha concentration shown better conditioning behaviour. The formulated shampoos were uniform, denser and stable similar as that of marketed shampoo. The formulated herbal powder shampoos has an excellent cleansing, detergency, conditioning and foaming effect, is suitable for regular hair and has acceptable pH and organoleptic stability characteristics.

KEYWORD: Castor, Henna, Reetha, Neem, Tulsi.

I. INTRODUCTION OF SHAMPOO

Hairs are the integral part of human beauty. People are using herbs for cleaning, beautifying and managing hair since the ancient era. Whereas the hair has been trimmed, shaped and even colored since the most ancient times, relatively little emphasis has been placed on the process of cleaning it.^[1,2] Only in this century has a real technology in the cleaning of the hair and scalp been developed. First came the mass distribution of cake soap and sanitary facilities to make bodily cleanliness and personal hygiene practice. Next came the specialization of branded shampoo products for the hair and scalp, offered in multiplicity of types and forms.^[3-5] Now, washing the hair and scalp with shampoo has become a nearly universal practice. Shampoos are probably the most widely used hair products today, based on synthetic ingredients as well as herbal ingredients. Shampoos are of various types, like powder shampoo, clear liquid shampoo liquid shampoo, lotion shampoo, solid gel

shampoo, medicated shampoo, liquid herbal shampoo etc.^[6] Dandruff is known to be controlled by fungi static ingredients in Anti-dandruff shampoos. Herbal formulation has growing demand in the world market. The natural remedies are more acceptable in market because it's safe and fewer side effect antidandruff shampoo and nutritional shampoo containing vitamin, amino acids proteinshydrolysate.^[7] Currently available treatment of dandruff include therapeutic use of zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and sulphur and coaltar derivatives. However, these agents show certain limitations, either due to poor clinical efficacy or due to the. Furthermore compliance issues, these drugs are unable to prevent recurrence.^[8] The synthetic shampoo contains cationic, anionic and non- anionic surfactant mix in this surfactant having good foaming character but its toxic and caused irritation of eye. Hard water the surfactants leave a deposit of sodium, calcium and magnesium salts on the

hair shaft. So, these synthetic shampoos are found to have side effects like drying effect on the hair. These shampoos leave the hair too dry to handle (or) comb, to avoid these problems, herbal shampoos will be useful.^[8,9]

The herbal shampoo powder was formulated using natural ingredients with Castor, Henna, Reetha, Neem Tulsi.

II. Herbal ingredient description

A. Reetha

There are many **Reetha benefits for hair**. Reetha powder is known as *Areetha* or soapnut powder. It is one of the ingredients mentioned in the ancient texts of Ayurveda, as it is known to improve hair and scalp health. Here are some benefits of using reetha in your hair care regime.

- **Safe and natural:** Chemical-heavy hair care product soften cause irreparable damage to your hair. If you want a safe and natural alternative to chemical-laden **shampoo**, reetha is your BFF! Reetha is gentle enough to use on all hair types, including color-treated hair, because it is safe and non-toxic.
- **Controls dandruff:** The antifungal and antibacterial properties can considerably reduce scalp infections and dandruff's intensity. Regular application soothes an itchy scalp and reduces inflammation.
- **Prevents hair loss:** Packed with antioxidant and antimicrobial properties, the reetha powder helps to promote a healthy scalp and prevent hair loss. In addition, when massaged on the scalp, reetha can help improve blood circulation to the hair follicles, promoting healthy growth.
- **Adds natural shine:** This magical ingredient can help to remove built-up impurities that often make your hair look dull and lifeless. The natural cleansing properties of reetha help restore your hair's natural shine, leaving it healthy and voluminous.
- **Conditions the hair:** Reetha powder contains natural conditioning properties that help to soften and visibly smoothen your hair, giving them a shiny and healthy look. It helps to detangle your hair and reduce frizz and thus making it easier to manage your hair.
- **Promotes hair thickness:** Reetha powder can help strengthen hair follicles, promoting thicker, fuller hair over time. It can also help prevent breakage and split ends, leading to thinner-looking hair.
- **Cleanses the scalp and hair:** Reetha powder can improve scalp health and prevent hair loss when used in a hair care regime. The natural surfactants in reetha can cleanse the scalp and hair from dirt, excess oil, and build-up without stripping away natural oils.



Fig. 1: Reetha for hair problems.

B. Castor

Many claims have been made about the benefits of using castor oil on your hair. However, there is little evidence to back up the anecdotes that say it improves hair health and growth.

Here are a few claims about castor oil and hair growth

- It increases blood flow to the scalp, which promotes healthier hair.
- Applying castor oil just once a month can boost hair growth up to five times the usual rate.
- It can moisturize a dry, irritated scalp.
- Castor oil's antibacterial and antifungal properties can reduce dandruff.
- It can help grow hair in other parts of the body, like the eyebrows and eyelashes.



Fig. 2: Castor for hair problems.

Some people claim that castor oil's benefits for hair are related to its potent antioxidant and anti-inflammatory properties. Antioxidants keep free radicals from damaging the body's healthy cells. This destructive process is called oxidative stress, and it's linked to many health effects, including hair loss.²Castor oil also contains omega-6 fatty acids a type of essential fatty acid that may promote hair growth and reduce inflammation in the hair follicle.

C. Neem

Neem oil is a natural byproduct of the neem tree, a type of evergreen that primarily grows in India. The oil is

pressed from the tree's fruits and seeds. This "wonder plant" has been widely used in traditional medicine and has demonstrated anti-inflammatory and antibacterial effects. But can using neem oil really result in a healthier scalp and hair? Here's what the research says, how to use it topically, and more. Anecdotal reports suggest that neem can.



Fig. 3: Neem for hair problems.

- **Condition your scalp**
- **Promote healthy hair growth**
- **Temporarily seal hair follicles**
- **Minimize grays**
- **Reduce dandruff**
- **Treat head lice**

Topical application delivers these nutrients directly to your hair, potentially resulting in healthier locks. It's also worth noting that vitamin E and other antioxidants can help skin cells regenerate. This may promote a healthier scalp, subsequently minimizing dandruff and resulting in healthier hair. Neem oil contains the active ingredient nimbidin. Some older research suggests that nimbidin can help suppress inflammation, which may make it useful in treating dermatitis, psoriasis, or other scalp irritation. Neem is also a known antifungal. In some cases, dandruff and irritation can result from yeast buildup on the scalp. Although more research is needed, there's reason to believe that topical application may help alleviate these symptoms. Researchers in one 2011 study found that neem seed extract successfully killed head lice larvae after 5 minutes of treatment and adult head lice after 10 minutes of treatment. This may be due to the oil's azadirachtin content. Azadirachtin can make it difficult for insects to grow and lay eggs by interfering with their hormones.

D. Basil or tulsi

Basil (*Ocimum basilicum*), also called sweet basil, annual herb of the mint family, grown for its aromatic leaves. Basil is likely native to India and is widely grown as a kitchen herb. The leaves of tulsi are used fresh or dried to flavour meats, fish, salads, and sauces; basil tea is a stimulant. **Basil oil** is also known as sweet basil essential oil. It is obtained from the leaves of the basil herb. It is used on the skin as it nourishes, repairs,

balances, calms, smoothens & brightens the complexion. It is used in hair as it cleanses, refreshes, hydrates & strengthens the strands. It enhances the immune function, protects from infection & stabilizes irregular menstruation.



Fig. 4: Tulsi for hair problems.

Benefits of basil for hair

The benefits of basil don't only pertain to its relevance in cooking, but also its immense levels of powerful nutrients that are amazing for your hair and skin. It is one of the most important herbs in Hindu Mythology has been used in ayurvedic medicine for thousands of years. With a host of benefits including anti-bacterial and anti-inflammatory properties that can help to heal a range of problems related to your skin, hair and overall wellbeing. It is an incredibly important ingredient in hair care products and can help to make your hair healthy. The benefits of Basil stem from its vast range of plant based chemicals. Packed with anti-oxidating flavonoids vicenin and orientin; essential oils like citronellol, eugenol, terpineol and limonene which have antibacterial and antiseptic properties; beta-carotene, zeaxanthin, lutein, vitamin A and Vitamin K which can help to heal your hair.

- **Hair loss:** Hair loss is generally attributed to sweat and itching of scalp. Applying a paste of basil leaves can help to cool down the scalp, reduce itching and improve the circulation in the scalp.
- **Dandruff and Dry scalp:** Dandruff and having dry scalp can be a major cause of hair loss. Using basil for your hair can help to improve the circulation in your scalp and can help to reduce itching and dandruff, thereby improving hair growth.
- **Strengthens hair:** Using basil for your hair can help to strengthen your hair, reduce the problem of hair thinning or your hair becoming brittle.
- **Boosts Immunity:** Basil can help to boost the immunity of your hair and make it healthy and hydrated due to the presence of anti-oxidants and immunity boosters.
- **Greying:** Basil can help to reduce greying, rejuvenate your hair and make it healthier due to the presence of anti-oxidants. It can help to prevent cell ageing and cell death.

E. Henna

Henna is one of the hair care ingredients that Indians have shared with the rest of the world. Indian women have been using henna for years, if not centuries, to care for their luscious locks. If you are allergic to chemical dyes and want to get rid of grey hair naturally, henna should be your go-to ingredient. It is loaded with tannins, a plant compound that is responsible for the rich colouring of tea. But you will discover multiple benefits of henna beyond dyeing. Let's learn more about how the henna powder is useful for your hair and how you can use it to get the maximum benefits.

➤ Improves scalp condition

Henna is the perfect ingredient to take care of your scalp and make it better. The cooling, antimicrobial, and antifungal properties of henna are great for improving the texture of your scalp. If your scalp feels itchy, you will get much relief from applying henna powder. Moreover, it can also help prevent scalp irritation and other fungal infections.

➤ Conditions your hair

If you use henna powder, it serves as a natural conditioner. The nourishing properties of henna will repair damage to the hair shaft. Henna will seal the hair cuticles to ensure that they can retain more moisture. Adding ingredients like yoghurt or egg with henna will improve the moisturizing properties of henna. It will also improve the strength and elasticity of your hair so that it can't break off easily. As a result, split ends in your hair will reduce, and your locks will start repairing naturally.

➤ Increases hair growth

Henna is one of the best ingredients for your scalp because it can improve the growth of hair follicles. Moreover, it can also make your hair follicles healthier. As a result, hair fall will be reduced, and the rate of hair growth will get a boost.

➤ Prevents dandruff



Fig. 5: Amla for hair problems.

Henna can help eliminate excess dirt or oil from your scalp. Applying henna to your hair regularly will fix the issue of dandruff. Moreover, it will also help prevent dandruff from coming back. Applying a paste of methi

seeds, henna, and mustard oil is the perfect way to keep dandruff at bay.

➤ Protects natural hair colour

Henna is often used by women who want to dye their hair. But the **best henna for hair** can improve the natural pigment of your hair. It will help prevent premature greying and also make your locks look thicker and more lustrous.

➤ Softens your hair

Henna comes with multiple nutritional properties that can transform your dry, damaged, and unhealthy hair. Once you apply henna to your hair, you will notice your locks becoming softer. As a result, your hair will also become more easily manageable.

III. Experiment or Formulation of herbal shampoo

All the plant material was dried and grinded. All the required herbal powders for shampoo preparation were weighed individually. The crude ingredients were collected and these ingredients were size reduced using hand driven mixer individually. All these fine ingredients were mixed thoroughly by mixer to form a homogenous fine powder. Then this fine powder was passed through sieve no:80, to get the sufficient quantity of fine powder. Then it was packed and labeled suitably.^[10,11] Prepared formulations of shampoos were subjected to following evaluation parameters.

A. Organoleptic evaluation

Organoleptic evaluation on the parameters like colour, odour taste and texture was carried out. Colour and texture was evaluated by vision and touch sensation respectively. For taste and odour evaluation a team of five taste and odour sensitive persons was formed and random sampling was performed.

B. General powder characteristic

General powder characteristics includes evaluation of those parameters which are going to affect the external properties (like flow properties, appearance, packaging criteria etc.) of the preparation, Characteristics evaluated under this section are powder form, particle size angle of repose and bulk density. Sample for all these evaluations were taken at three different level i.e. from top, middle and lower level.

C. Particle size

Particle size is a parameter, which could affect various properties like spread-ability, grittiness etc., particle size was determined by sieving method by using I.P. Standard sieves by mechanical shaking for 10 Min.

D. Angle of repose

It is defined as the maximum angle possible in between the surface of pile of powder to the horizontal flow. Funnel method

Required quality of dried powder is taken in a funnel

placed at a height of 6 cm from a horizontal base. The powder was allowed to flow to form a heap over the paper on the horizontal plane. The height and radius of the powder was noted and recorded the angle of repose (θ) can be calculated by using the formula. Required amount of dried powder is placed in a cylindrical tube open at both ends is placed on a horizontal surface. Then the funnel should be raised to form a heap. The height and radius of the heap is noted and recorded. For The above two methods, the angle of repose (θ) can be calculated by using the formula. $\theta = \tan^{-1}(h/r)$

Where,

θ –Angle of repose, h–height of the heap, r –Radius of the base

E. Bulk density

Bulk density is the ratio between the given mass of a powder and its bulk volume. Required amount of powder is dried and filled in a 50 ml measuring cylinder up to 50 ml mark. Then the cylinder is dropped onto hard wood surface form a height of 1 inch at 2 second interval. The volume of the powder is measured. Then powder is weighed. This is repeated to get average values.

F. Tapped density

The tapped density is an increased bulk density attained after mechanical tapping a container containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped for 1 min and volume or mass reading are taken until little further volume or mass change was observed. It was expressed in gram per cubic centimeter

G. Moisture content

Moisture content in the formulation is very important as it contains herbs which are liable to be attacked by weather. 2gm of powder was taken and kept in an oven and dried up to two Constant reading and % moisture content was calculates as w/w.

IV. Physico chemical evaluation

A. pH: pH affect the pharmaceutical consideration as well as it affect the effect of shampoo on hairs. 1gm of powder shampoo was taken and 9ml of distilled water was added to it. pH of the resulting

solution was calculated using pH meter at 37⁰c.

B. Wash-ability: Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

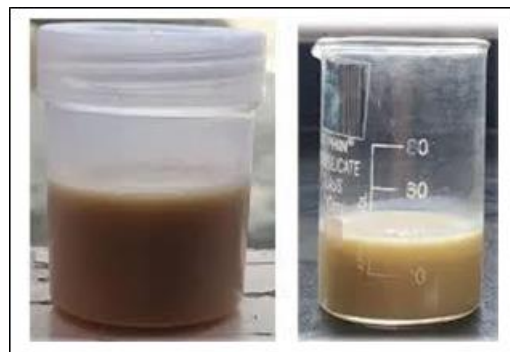


Fig. 6: Prepared herbal shmpoo.

C. Solubility: Solubility is defined as the ability of the substance to soluble in a solvent. One gram of the powder is weighed accurately and transferred into a beaker containing 100ml of water. This was shaken well and warmed to increase the solubility. Then cooled and filter it, the residue obtained is weighed and noted.

D. Skin irritation test: The skin irritation tests revealed that the herbal shampoo powder shows no harmful effect on skin. This is due to the absence of synthetic surfactants. Most of the synthetic surfactants produce inflammation of the eyelid and corneal irritation. But in this formulation of herbal shampoo powder, the uses of all ingredients are obtained naturally. So it does not produce any harmful effect on skin.

E. Ash value: Total ash content Ash value is calculated to determine the inorganic contents which are characteristic for a herb. About 2gm of powder drug was taken in silicon dish previously ignited and weighed. Temperature was increased by gradually increasing the heat not exceeding to red colour. After complete burning, ash is cooled and weighed.

V. Evaluation and Physio-chemical parameters.

A. Results of Physio-chemical parameters.

No.	Parameter	Value Obtained (%w/w)				
		Name of Drugs				
		Castor	Reetha	Neem	Heena	Tulsi
1	Foreign Organic matter	1.09	1.00	1.52	1.32	1.25
2	Total Ash value	1.10	1.69	10.9	9.4	12.4
3	Acid Insoluble Ash value	1.02	0.24	0.96	2.51	4.22
4	Alcohol soluble extractive	1.20	6.24	6.51	5.07	2.23
5	Water Soluble extractive	6.25	12.61	15.47	16.23	7.50
6	Loss on drying	7.90	6.82	10.5	9.24	8.25

B. Evaluation of physiochemical parameters.

No.	Name of test	Aqueous extract				
		Name of drugs				
		Castor	Reetha	Neem	Heena	Tulsi
1	Alkaloids.					
a	Mayer's	+	-	+	-	-
b	Hagar's	+	-	+	-	-
c	Wagner's	+	-	+	-	+
2	Carbohydrates					
a	Fehling's	+	+	+	+	+
b	Benedict's	+	+	+	+	-
c	Molish's	+	+	+	-	-
3	Glycosides					
a	Borntrager's	-	-	-	+	-
b	Legal's	-	-	-	-	-
c	Keller-Killani	-	-	+	-	-
4	Saponin					
a	Foam	+	+	+	-	+
5	Tannins and Phenolic compound					
a	FeCl ₃ Solution	+	-	+	+	+
b	Lead Acetate	+	-	+	+	+
c	DiHNO ₃	-	-	+	+	+
6	Flavonoids					
a	Shinoda	+	-	+	+	+
b	Lead Acetate	+	-	+	+	+
c	Sodium Hydroxide	+	-	+	+	+
7	Phytosterol					
a	Salkowaski	-	-	-	-	-
8	Protein and Amino Acid					
	Xanthprotic	+	+	-	-	-
	Biuret	-	-	-	-	-
	Ninhydrin	-	-	-	+	+

C. Results of Phyto-chemical Parameters of ethanol extract.

No.	Name of Test	Ethanol Extract				
		Name of Drugs				
		Castor	Reetha	Neem	Heena	Tulsi
1	Alkaloids.					
a	Mayer's	+	-	+	-	-
b	Hagar's	+	-	+	-	+
c	Wagner's	+	-	+	-	-
2	Carbohydrates					
a	Fehling's	+	-	+	+	+
b	Benedict's	+	+	+	+	-
c	Molish's	-	+	+	-	-
3	Glycosides					
a	Borntrager's	-	-	+	+	-
b	Legal's	-	-	+	-	-
c	Keller-Killani	-	-	+	-	-
4	Saponin					
a	Foam	-	+	+	-	-
5	Tannins Phenolic compound a					
a	FeCl ₃ Solution	+	-	+	+	-
b	Lead Acetate	-	-	+	+	-
c	DiHNO ₃	-	-	+	+	-
6	Flavonoids					
a	Shinoda	+	+	+	+	+
b	Lead Acetate	+	+	+	+	+

c	Sodium Hydroxide	-	-	+	+	+
7	Phytosterol					
a	Salkowaski	-	-	-	-	-
8	Protein and Amino Acid					
	Xantho protic	-	-	-	-	-
	Biuret	-	-	-	-	-
	Ninhydrin	-	-	-	+	+

D. Result of preformation of herbal shampoo powder. (Antimicrobial Activity)

Batch No.	Zone of Inhibition		
	Microorganism		
	Bacillus Subtills	Escherichia Coli	M.Furfur
1	15	13	14
2	13	12	17
3	17	16	23
4	19.5	18	24
5	16	13.5	19
6	16.5	15	20.5
7	12	10.5	10
8	12.5	11	11.5
9	13	14.5	14
10	14	15	18
11	13	12	12
12	13.5	13	13.5
13	12.5	11.5	12.5
14	10	11	14
15	16	14.5	18
16	17	15.5	19.5
17	13	12.5	15.5
18	16.5	14	16

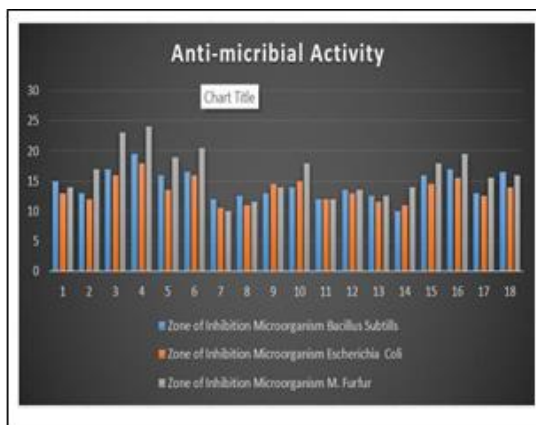


Fig. 7: Comparison of Antimicrobial activity.

E. Result of Pre-formation of herbal shampoo Powder. (Cleansing Action)

Batch No.	Cleansing Action
1	23.12 %
2	30.15 %
3	35.37 %
4	44.32 %
5	29.20 %
6	33.69 %
7	19.23 %
8	22.84 %
9	13.55 %
10	37.12 %

11	25.34 %
12	28.75 %
13	21.69 %
14	27.12 %
15	20.20 %
16	41.20 %
17	27.14 %
18	31.34 %



Fig. 8: Cleansing action of herbal shampoo.

F. Organoleptic evaluation of general powder.

No	Organoleptic evaluation	Result			
		Batch No.			
		3	4	6	
1	Colour	Greenish	Greenish	Greenish	
2	Odour	Characteristic	Characteristic	Characteristic	
3	Texture	Fine and Smooth	Fine and Smooth	Fine and Smooth	
General Powder Characteristic					
1	Angle of Repose	Funnel Method	32.65	35.45	32.08
		Open end Cylindrical method	31.46	34.99	31.38
2	Bulk Density	0.22g/cm ²	0.29g/cm²	0.34g/cm ²	
3	Tapped Density	0.35g/cm ²	0.32g/cm²	0.38g/cm ²	

G. Particle size analysis of formulation.

Batch No.	Sieve No.				
	44	60	80	100	120
3	0.140	1.618	1.200	1.308	0.369
4	0.963	2.164	1.700	1.232	0.394
6	1.056	1.819	1.086	0.713	0.161

H. Physicochemical properties.

No	Physico-chemical Evaluation	Result			
		Batch No.			
		3	4	6	
1	Extractive Values	Alcohol Soluble	17.54 % w/w	18.60% w/w	16.45% w/w
		Water soluble	14.08% w/w	13.78% w/w	13.15% w/w
2	Ash Value	Total ash	4.56% w/w	4.18% w/w	4.25% w/w
		Acid soluble ash	1.22% w/w	1.39% w/w	1.09% w/w
3	Moisture content	2.38 %	2.61%	2.02%	
4	pH	5.35	5.69	5.41	
5	Washability	Easily Washable	Easily Washable	Easily Washable	
	Cleansing action	35.37 %	44.32%	33.69%	
6	Foaming capacity	Foam Height	200 ml	220 ml	190 ml

7	Foam Retention	34 ml	40 ml	30 ml
8	Dirt dispersion	Moderate	Light	Moderate
9	Wetting Time	2 minutes 20 sec	2 minutes 10 sec	2 minutes 35 sec

I. Antimicrobial activity of herbal powder shampoo extract.

Micro organism	Zone of inhibition in mm						
	Concentration of Plant Extract (mg/ml)				standard		control
	100	200	300	400	Azithromycin (mg/ml)	Fluconazole (mg/ml)	water
B. Subtilis	14.5	16.0	18.5	19.5	23	-	-
E. Coli	13.0	15.5	17.0	18.0	21	-	-
M. furfur	19.5	21.0	22.5	24.0	-	25	-

G. Anti-Microbial Activity against bacteria and fungi.

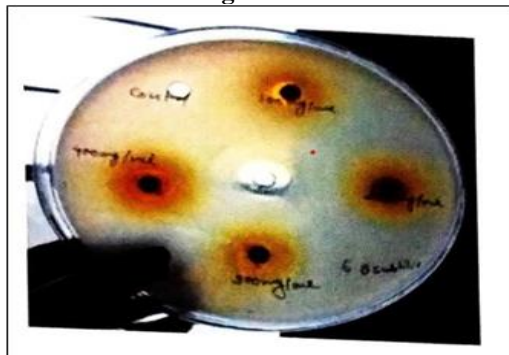


Fig. 9: Zone of Inhibition of Extract and Standard against B. Subtilis.



Fig. 10: Zone of Inhibition of Extract and Standard against E. Coli.

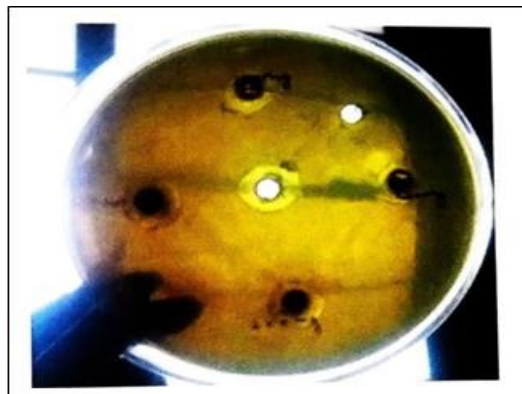


Fig. 11: Zone of Inhibition of Extract and Standard against M. Furfur.

H. Minimum inhibitory concentration of extract.

No.	Name of Microorganism	Minimum inhibitory concentration (mg/ml)
1	B. Subtilis	100
2	E.Coli	100
3	M. Furfur	100

I. Skin irritation test.

Sr. No.	Time	Reaction
1	Control	No reaction
2	24 Hr.	No reaction
3	48Hr.	No reaction
4	72 Hr.	No reaction

J. Dataanalysis

Various computations for the current optimization study using Response Surface Methodology (RSM) were carried out, employing the Design Expert software (Version 8.0.0). Statistical second-order model including interaction and polynomial equation were generated for all the response variables using multiple linear regression analysis (MLRA). The general form of model is represented as in equation (1);(1) Where β_0 , the intercept is arithmetic average of all quantities outcomes of 9 runs, β_1 , to β_3 are the coefficient computed from the observed experimental values of the independent variables(s). The term X_1X_2 and X_i^2 ($i = 1, 2$) are the interaction and polynomial terms, respectively. The statistical validity of the polynomials was established on

the basis of Yare's ANOVA provision in the design expert software. Subsequently, feasibility as well as grid search was performed to locate the composition of optimum formulations. Also, three dimension response surface graphs were generated by the design expert software.

K. Designofexperiments

Factorial design was applied to design the experiments. On the basis Preliminary trials concentration of Castor, Reetha. Whereas Antifungal activity an Cleansing, were kept as dependent variables. Formulations F1 to F9 were prepared using Castor and Reetha concentration at three different levels. The summary of the formulations is shown in Table Indicate variables.

Independent variables	Dependent variables
X_1 =Castor	Y_1 =Antifungal Activity
X_2 =Reetha	Y_2 =Cleansing Activity

K. Factor and Levels with their real value.

Factor	Level(-1)	Middle Level(0)	High Level(1)
Castor (g)	6 gm	9 gm	12 gm
Reetha (g)	6 gm	9 gm	12 gm

L. Results of optimized batch of herbal powder.

Std	Run	Factor 1 Castor(g)	Factor2 Reetha (g)	Response 1 Antifungal Activity(mm)	Response 2 Cleansing (%)
1	1	+1.00	+1.00	23.5 ±0.62	45.66±0.33
7	2	-1.00	0.00	15.5±0.40	24.64±0.53
8	3	0.00	-1.00	16.5±0.40	28.34 ±0.48
5	4	-1.00	-1.00	13.5±0.23	22.22±0.81
4	5	1.00	-1.00	19.0±0.23	30.20±0.45
9	6	-1.00	+1.00	16.5±0.23	38.83±0.46
3	7	0.00	1.00	22.0±0.40	44.32 ±0.43
2	8	1.00	0.00	21.5±0.62	41.50±0.48
6	9	0.00	0.00	18.5±0.23	35.42±0.47

(+1)Higher Value (0) Middle Value(-1) Lower Value

R1= Antifungal Activity

R2=Cleansing Activity + S.D. N=3

M. Antifungal activity in mm.

Batch	Zone of Inhibition				Standard	Control
	100 mg	200 mg	300 mg	400 mg		
F1	17.5	19.5	21.0	23.5	24.5	-
F2	14.0	15.5	17.0	19.0		
F3	13.5	14.0	16.0	18.5		
F4	12.5	14.0	15.5	16.5		
F5	9.0	10.0	11.0	13.5		
F6	11.0	12.5	14.0	15.5		
F7	13.5	14.0	15.5	16.5		
F8	15.5	16.0	18.5	21.5		
F9	16.5	18.0	19.5	22.0		

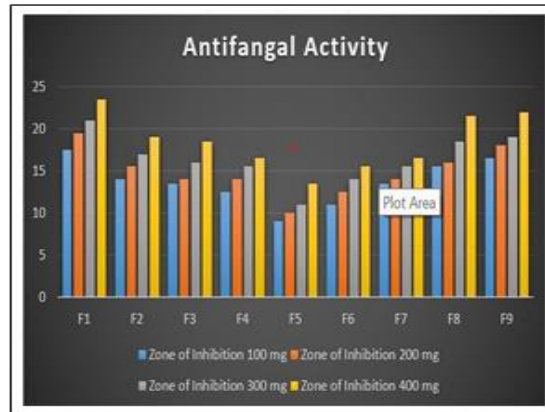


Fig. 12: Antifungal Activity in mm x axis represents the Batch No and Y axis represent the zone of inhibition.

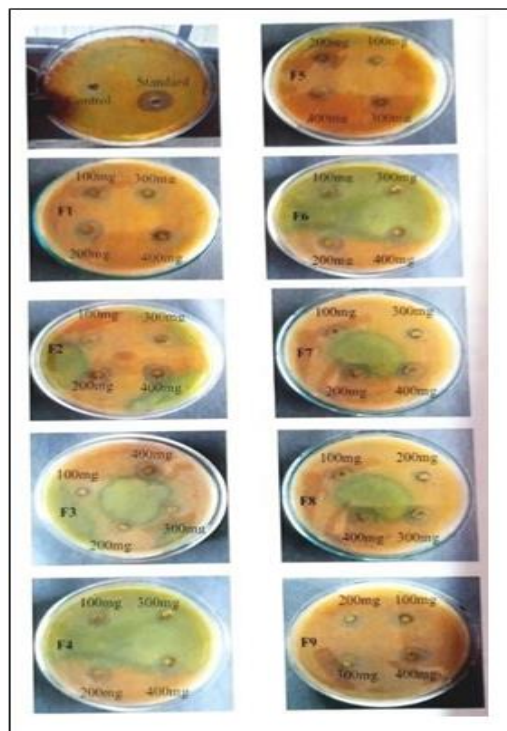


Fig. 12: Zone of inhibition of antifungal activity of formulation.

N. Evaluation of Dependent Variables and mathematical modeling.

Significant values (Probable values)

No.	Significant values (Probable values)	
1	Antifungal Activity	0.0144
2	% Cleansing	0.0366

Significant summary of ANOVA for the response parameters.

Source	Sum of Squares	d.f.	Mean Squares	F-Values	P-Values Probe>F
Antifungal Activity(mm)					
Model	48.67	5	9.73	21.90	0.0144 (S)
X ₁	32.67	1	32.67	73.50	0.00333(S)
X ₂	13.50	1	13.50	30.37	0.0118(S)
X ₁ X ₂	0.000	1	0.000	0.000	1.00000(NS)
X ₁ ²	2.00	1	2.00	4.50	0.1240(S)
X ₂ ²	0.50	1	0.50	1.12	0.36687(S)
Cleansing%					
Model	564.32	5	112.86	11.31	0.0366(S)
X ₁	166.85	1	166.85	16.73	0.0264(S)
X ₂	385.28	1	385.28	38.63	0.084 (S)
X ₁ X ₂	0.35	1	0.35	0.035	0.0084(NS)
2X ₁	9.50	1	9.50	0.95	0.4010(S)
2X ₂	2.33	1	2.33	0.23	0.6618(NS)

X₁, X₂ represent Castor and reetha, respectively X₁ X₂, X₁², and X₂² are the interaction effects. S. indicates significant. D.F. indicates degree of freedom For the calculated Antifungal activity, Cleansing F- values are 21.90 and 11.31 respectively as shown in table 8.16. Hence it can be concluded that the variables selected contributes significantly in the regression of measured responses Y₁ and Y₂.

From equation 1 it can be concluded that positive coefficient of both X and X₂ indicated increase in Antifungal activity(Y₁) of herbal powder shampoo with

increase in the Castor and Reetha concentration. When the both variables at high level their interactive effect were significant. For Antifungal activity equation 2 was obtained from design model. Positive coefficient of both X₁ and X₂(increase in Castor and Reetha concentration) indicated increase of Antifungal activity the herbal powder shampoo. These effects can be further explained by response plots and contour plots generated using equation 1, 2 and 3. The response and contour plots are shown in fig.

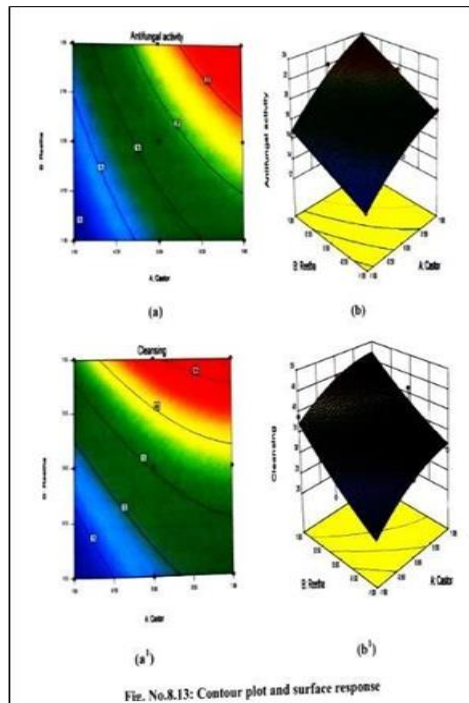


Fig. No. 8.13: Various plots showing influence of (X₁) and (X₂) on the MIC(a) Contour plot(a').

Response surface plot cleansing (b) Contour plot (b')
Response surface plot.

The response surface plot (8.13 a) and contour plot (8.13 a') indicated the relatively increased in Antifungal activity with increased in castor and reetha

concentration. With increase in castor and reetha concentration Antifungal activity was increased and this can be confirmed from equation 1. At different levels (-1, 0, 1) of castor and reetha concentration was increased, the cleansing was increased. The surface response plot (8.13 b) and contour plot (8.13 b¹) showed increase in response (Y₂) cleansing as castor and reetha concentration was increased from lower to higher level. This may be due to the higher cleansing of herbal powder shampoo as reetha level increases.

Search for optimum formulation of herbal powder shampoo

The results for the feasibility to find the suitable region for further location of optimum formulation are presented in table 8.16.1 the criteria for selection of suitable feasible region was primarily based upon the value of Antifungal activity, Cleansing One formulation was selected on the basis of the following criteria.

No.	Response	Region
1	Anti-Fungal Activity in mm	23.5-13.5 mm
2	Cleansing Activity in %	22.22-45.66 %

O. Disairability and Overlay point for searching optimum formulation of Herbal Powder Shampoo.

Sr. No.	Factor	Optimized ratio	Calculated Quantity
1	X ₁	0.70	18.50 gm
2	X ₂	-0.55	5.184 gm

Batch	Composition		Response	Predicted value	Actual Value	% Error
	X ₁	X ₂				
Optimized Batch	0.70	-0.55	Antifungal Activity	19.61	19.0	0.31
			Cleansing Activity	33.94	34.1	0.071

P. Antifungal activity of optimized batch.

Sr. No	Optimized Batch
0	0
1	17.5 ± 0.5
2	19.5 ± 0.5
3	21.5 ± 1.0
4	22.5 ± 1.0

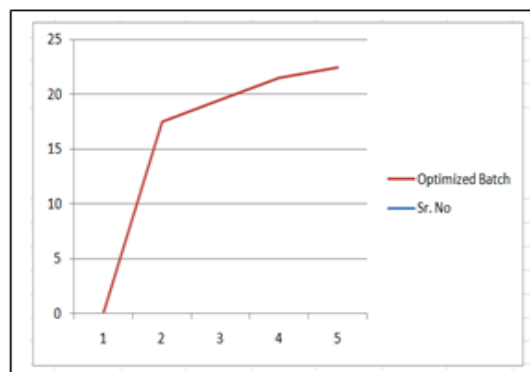


Fig. 13: Optimized batch.

RESULT AND DISCUSSION

Herbal powder shampoo was prepared using Castor, Henna, Reetha, Neem Tulsi in different composition. These formulations were prepared using are Castor, Henna, Reetha, Neem Tulsi has been reported for hair growth and conditioning. The various quality control parameters were checked. All parameter gives favorable result. The result obtained on present study shows that the active ingredients of these drugs when incorporated

in shampoo gives more stable products with good aesthetic appeal. The pH of the shampoo 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 minimizing damages to the hair. Such results are estimated out of a formulation to establish strong results for the usage and good results of the product. Though the

product is in dry form inspite has wonderful wetting capacity and being dry is very good for the storage. The evaluation parameters like Organoleptic evaluation, General powder Characters, Physicochemical Evaluation, Cleaning action, foaming, wetting agent, Nature of hair after wash was carried out and was found to be within the standard range.

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