FORMULATION AND EVALUATION OF HERBAL ANTI-DANDRUFF SHAMPOO USING DRUG DESIIGN SOFTWARE

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ABSTRACT

Herbal based cosmetic formulations have gained for safety, biocompatibility, and efficacy in managing scalp disorder. Dandruff linked to Malassezia overgrowth, requires long-term treatment. This study developed, optimized, and evaluated a polyherbal anti-dandruff shampoo using extracts of *Piper nigrum, Moringa oleifera* and *Momordica charantia* for their antifungal, anti-inflammatory, antioxidant and antimicrobial properties. Extracts were prepared via decoction and maceration. Seventeen formulations (F1-F17) were developed with varying extract concentrations and optimized using Box-Behnken Design. Five promising ones underwent comprehensive evaluation for physicochemical properties: colour, clarity, odour, pH, viscosity, surface tension, solid content, wetting time, foam capacity, foam stability, and antifungal activity. Formulation F16 excelled with pH~6.9, optimal viscosity, low surface tension, excellent foaming/stability, effective wetting and antifungal activity. It offers dandruff control, hair conditioning, and scalp health without irritation, positioning polyherbal shampoos as safe alternatives to synthetics.

Key words: *Malassezia, Piper nigrum, Moringa oleifera, Momordica charantia, Box-Behnken Design, Optimization.*

INTRODUCTION

Cosmetics are formulations intended for external application to human body for the purpose of cleansing, beautifying, protecting and maintaining the health of skin and hair. Among various cosmetics products hair care formulations such as shampoo play a vital role in maintaining scalp hygiene and improving hair quality. In recent years, there has been a growing interest in herbal cosmetics due to their safety, compatibility with biological systems, and minimal adverse effects compared to synthetic counterparts. Dandruff is a common chronic scalp disorder characterized by flaking, itching, and irritation often associated with the proliferation if lipophilic fungi such as *Malassezia* species. Although not life-threatening, dandruff can affect personal appearance and quality of life. Conventional anti-dandruff shampoos typically contain synthetic agents such as zinc pyrithione or ketoconazole; however their prolonged use may lead to side effects such as scalp irritation, dryness, and resistance. This led to increased demand for safer and more effective herbal alternatives. Herbal formulations utilize plant-derived ingredients rich in bioactive compounds such as flavonoids, alkaloids, and phenols which exhibit antifungal, anti-inflammatory, antioxidant and conditioning properties. Medicinal plants such as Piper nigrum, Moringa oleifera and Momordica charantia have been used for hair acre due to their ability to combat microbial growth, soothe scalp irritation, and promote healthy hair growth.

In addition to selecting effective herbal ingredients, formulation optimization crucial to ensure desirable physicochemical properties and therapeutic efficacy. Modern approaches such as statistical design software facilitates systematic optimization of formulations by evaluating the influence of various components and their interactions. This enhances the quality, stability and performance of final product. Therefore, the present study focuses on the formulation and evaluation of an optimized polyherbal anti-dandruff shampoo using selected plant extracts. This study aims to develop a stable, effective, and safe herbal formulation with significant antifungal activity while maintaining desirable cosmetic properties. This work also highlights the potential of integrating traditional herbal knowledge with modern formulation techniques for development of effective cosmeceutical product.

LIST OF MATERIALS USED

Table :1 List of materials used for preparation of formulation

Sl. No.	List of materials	Role
1	Pepper	Antifungal
2	Moringa leaves	Antioxidant
3	Bitter gourd	Anti-inflammatory
4	Potassium hydroxide	Saponifying agent
5	Coconut oil	Emollient
6	Boric acid	Buffering agent
7	EDTA	Chelating agent
8	Sodium benzoate	Preservative
9	Tween 80	Solubilizing agent
10	Tea tree oil	Fragrance

EXTRACTION OF PEPPER

Pepper was extracted using Soxhlet method. Pepper extract was prepared by dry and grind pepper into coarse powder. Load the thimble with pepper, assemble the Soxhlet, add water to round bottom flask and attach to condenser. Gently heat the flask so the water vaporizes and drip on to the pepper, soaking it. Once thimble fills, solvent siphon backs into the flask, concentrating the extract. Repeat the cycle for several hours. Evaporate the water to get crude extract.



Figure No:1 Extraction of pepper

EXTRACTION OF MORINGA LEAVES

Moringa leaves was extracted using maceration method. Moringa leaves are dried and crushed into fine powder. Powder is placed in container and add water, stir the mixture occasionally then allow the mixture to sit for a period of time such as 3 days for maceration. Then filter it.

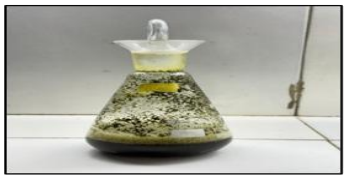


Figure No:2 Extraction of moringa leaves

EXTRACTION OF BITTER GOURD

Wash fresh bitter gourd thoroughly and remove seeds. Blend the chopped gourds with water. Pour the mixture through a strainer to separate the pulp, leaving the smooth juice.



Figure No: 3 Extraction of bitter gourd

FORMULATION OF SHAMPOO

Table No:2 Formulation of shampoo

Ingredients	KOH [10%] mg	Coconut oil ml	Boric acid mg	EDTA mg	Water	Herbal extract ml
F1	1.06	3.2	qs	0.02	qs	5
F2	1.06	3.2	qs	0.02	qs	30
F3	1.06	3.2	qs	0.02	qs	5
F4	1.06	3.2	qs	0.02	qs	30
F5	1.06	3.2	qs	0.02	qs	5
F6	1.06	3.2	qs	0.02	qs	30
F7	1.06	3.2	qs	0.02	qs	5
F8	1.06	3.2	qs	0.02	qs	30
F9	1.06	3.2	qs	0.02	qs	5
F10	1.06	3.2	qs	0.02	qs	30
F11	1.06	3.2	qs	0.02	qs	17.5
F12	1.06	3.2	qs	0.02	qs	17.5
F13	1.06	3.2	qs	0.02	qs	17.5
F14	1.06	3.2	qs	0.02	qs	17.5
F15	1.06	3.2	qs	0.02	qs	17.5
F16	1.06	3.2	qs	0.02	qs	17.5
F17	1.06	3.2	qs	0.02	qs	17.5

EXPERIMENTAL DESIGN

BOX-BEHNKEN DESIGN

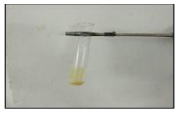
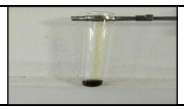
The Box-Behnken Design is a statistical experimental design often used for optimization in formulation development, because it requires fewer experiments compared to full factorial designs.

Table No:3 Observation values of response

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
		A: Conc of herbal extract ml	B: Stirring speed rpm	C: Conc of foaming agent ml	Viscosity cpm	Foaming index
12	1	5	40	1	3200	330
1	2	30	40	1	3150	345
3	3	5	100	1	3180	335
13	4	30	100	1	4000	410
10	5	5	40	5	4200	435
9	6	30	40	5	4180	418
17	7	5	100	5	4250	440
15	8	30	100	5	3250	348
14	9	5	70	3	4200	415
2	10	30	70	3	4150	410
8	11	17.5	40	3	3300	350
5	12	17.5	100	3	3170	342
11	13	17.5	70	1	3950	395
16	14	17.5	70	5	4600	465
7	15	17.5	70	3	4450	440
6	16	17.5	70	3	4650	455
4	17	17.5	70	3	4520	445

PHYTOCHEMICAL SCREENING

Table No:4 Phytochemical screening

SL.NO	Phytochemical constituents	Observation	Inference	Picture
1	Saponins	Persistent and stable foam formation	Presence of saponins	
2	Tannins	Formation of cloudy precipitation	Presence of tannins	
3	Phenols	Formation of violet colour	Presence of phenols	
4	Alkaloids	Formation of brown precipitate	Presence of alkaloids	

RESULT AND DISCUSSION

ANOVA FOR QUATRATIC MODEL- VISCOSITY

Response 1: Viscosity

Table No: 5 Anova for viscosity

Source	Sum of Squares	df	Mean Square	F-Value	P-Value	
Model	4.24E+06	9	4.71E+05	3.91	0.043	Significant
A-conc of herbal extract	9000	1	9000	0.0747	0.7925	
B-stirring Speed	3240	1	3240	0.0269	0.8744	
C-conc of foaming agent	9.00E+05	1	9.00E+05	7.47	0.0292	
AB	1512.5	1	1512.5	0.0126	0.9139	
AC	4.01E+05	1	4.01E+05	3.32	0.1111	
BC	3.66E+05	1	3.66E+05	3.03	0.1251	
A^2	8847.41	1	8847.41	0.0734	0.7942	
B^2	2.09E+06	1	2.09E+06	17.32	0.0042	
C^2	66432.31	1	66432.31	0.5513	0.4819	
Residual	8.44E+05	7	1.21E+05			
Lack of Fit	8.23E+05	5	1.65E+05	15.98	0.0599	not significant
Pure Error	20600	2	10300			
Cor Total	5.08E+06	16				

Table No: 6 Fit summary of viscosity

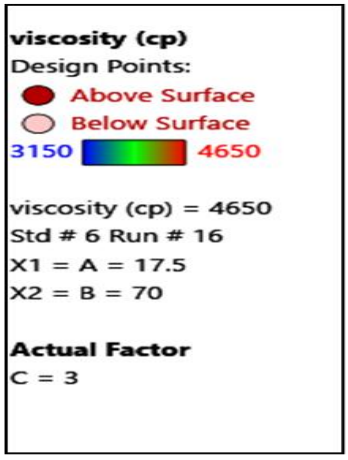
Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.4459	0.0269	-0.0098	-0.4155	
2F1	0.5457	0.024	-0.071	-2.0049	
Quadratic	**0.0159**	**0.0599**	**0.06206**	**-1.3086**	**Suggested**
Cubic	0.5978	0.0255	0.5712	-120.116	**Aliased**

Table No: 7 Lack of fit

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	4.15E+06	11	3.77E+05	36.61	0.0269	
2F1	3.38+06	8	4.23E+05	41.03	0.024	
Quadratic	**8.23E+05**	**5**	**1.65E+05**	**15.98**	**0.0599**	**Suggested**
Cubic	3.88E+05	1	3.88E+05	37.66	0.0255	Aliased
Pure Error	20600	2	10300			

Table No: 8 Fit statistics

Std. Dev.	347.13	**R^2**	0.834
Mean	3905.88	**Adjusted R^2**	0.6206
C.V.%	8.89	**Predicted R^2**	-1.3086
		Adeq Precision	6.8674



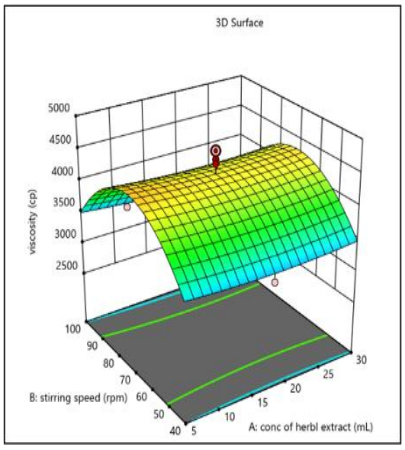


Figure No:4 3D surface plot for viscosity

Response: 2 Foaming index

Table No: 9 Anova for foaming index

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	30061.9	9	3340.21	4.58	0.0286	Significant
A-conc of herbal extract	57.6	1	57.6	0.079	0.7867	
B-stirring Speed	0.9	1	0.9	0.0012	0.9729	
C-conc of foaming agent	8468.1	1	8468.1	11.62	0.0113	
AB	28.12	1	28.12	0.0386	0.8498	
AC	4950.12	1	4950.12	6.79	0.0351	
BC	2278.13	1	2278.13	3.13	0.1204	
A^2	0.2812	1	0.2812	0.0004	0.9849	
B^2	11733.14	1	11733.14	16.1	0.0051	
C^2	851.18	1	851.18	1.17	0.3157	
Residual	5101.63	7	728.8			
Lack of Fit	4984.96	5	996.99	17.09	0.0562	not significant
Pure Error	116.67	2	58.33			
Cor Total	35163.53	16				

Table No:10 Fit Summary for foaming index

Source	Sequential P-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.2908	0.0239	0.0677	-0.3508	
2F1	0.3437	0.0239	0.1182	-1.301	
Quadratic	**0.0194**	**0.0562**	**0.6684**	**-0.8527**	**Suggested**
Cubic	0.6575	0.0218	0.0218	-115.648	**Aliased**

Table No: 11 Lack of Fit

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	26520.26	11	2410.93	41.33	0.0239	
2F1	19263.89	8	2407.99	41.28	0.0239	
Quadratic	**4984.96**	**5**	**996.99**	**17.09**	**0.0562**	**Suggested**
Cubic	2585.56	1	2585.56	44.32	0.0218	Aliased
Pure Error	116.67	2	58.33			

Table No:12 Fit Statistics

Std. Dev.	27	**R^2**	0.8549
Mean	398.71	**Adjusted R^2**	0.6684
C.V.%	6.77	**Predicted R^2**	-0.8527
		Adeq Precision	7.9679

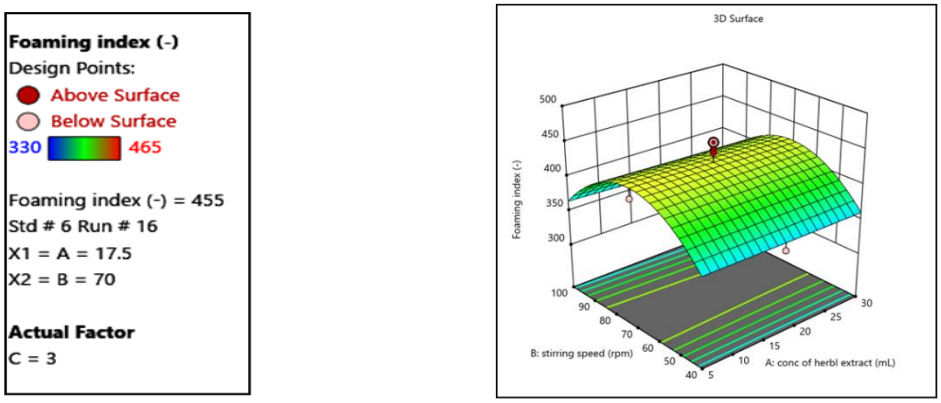


Figure No:5 3D surface plot for foaming index

We obtained F16 as our best formulation from optimization which have the foaming index 455, and viscosity 4650c

ORGANOLEPTIC EVALUATION

Table No:13 Organoleptic evaluation

SL. NO.	PARAMETER	OPTIMIZED FORMULATION
1	Colour	Reddish brown
2	Odour	Pleasant
3	State	Liquid
4	Consistency	Light watery



Figure No:6 Formulation of Herbal Anti-dandruff Shampoo

Table No:13 Evaluation parameters Data

Parameter	Optimized formulation (F16)	Mean±SD
pH	Good	5.17±0.02
Viscosity	Good	4650±1
Percentage of solid content	Good	25±0.6
Foam ability and foam stability	Good	455±1
Wetting time	Good	148±2.1

ANTI FUNGAL ACTIVITY

Antifungal activity of sample was assessed using agar well diffusion method to evaluate its biological efficacy. Samples diffuse into the agar medium on plates freshly inoculated with test fungi, forming circular zones of inhibition amid conflict growth; these zones diameter measured in millimeters. Potato dextrose agar plates were swabbed with over-night grown *Candida albicans*, 10 mm wells were punched, and varying sample volumes such as 25,50, and 100 µl were added. Zones were measured post-overnight room temperature incubation and compared to the standard antimycotic clotrimazole (NCCLS,1993).

Table No:14 Zone of inhibition of formulated herbal anti-dandruff shampoo

Sample	Concentration	Zone of inhibition (mm)
Herbal Anti-Dandruff Shampoo (F16)	Clotrimazole (100µg)	24
	25µl	26
	50µl	31
	100µl	38

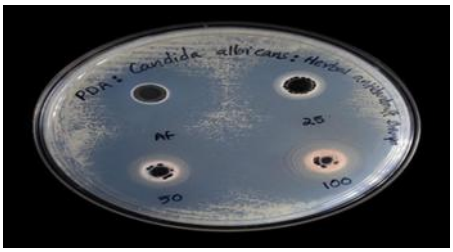


Figure No:7 Test for antifungal activity by ager well diffusion method

COMPARATIVE EVALUATION OF MARKETED AND FORMULATED SHAMPOO

Table No:15 Comparative study of Anti-Dandruff shampoo

Parameter	Marketed shampoo	Formulated shampoo
pH	5.5-6.5	5.17-6.0
Viscosity	800-6000cp	3150-4650cp
Foaming index	480	455
Wetting time	24-187sec	45-150sec
Solid content	20-30%	24-28%

The comparative evaluation indicates that the marketed anti-dandruff shampoo demonstrates relatively better performance across key physicochemical parameters. Although the formulated shampoo meets standard quality requirements, the marketed product shows improved pH suitability, foaming efficiency, wetting characteristics, and formulation consistency.

Comparative study of Antifungal Activity



Figure No:8 Comparative study of antifungal activity of Candida albicans

Table No: 16 Zone of inhibition of formulated and marketed shampoo

SAMPLE	CONCENTRATION	ZONE OF INHIBITION mm
Formulated anti-dandruff shampoo	Clotrimazole (100µg)	24
	25µl	26
	50µl	31
	100µl	38
Marketed anti-dandruff shampoo	Ketoconazole (100µg)	25
	25µl	28
	50µl	34
	100µl	40

Result: The zone inhibition is increased with increasing concentration of formulated shampoo, indicating a concentration-dependent antifungal effect. However, the marketed shampoo produced a larger zone of inhibition, demonstrating superior antifungal efficacy against Candida albicans.

CONCLUSION

Herbal anti-dandruff shampoos formulated with natural extracts of pepper, moringa leaves, and bitter gourd were developed to cleanse the scalp, support hair health, and control dandruff and hair loss associated with fungal infections. Among the tested formulations (F1-F17), F16 showed the highest antifungal activity against *Candida albicans*. Since dandruff is mainly caused by *Malassezia furfur* and sometimes *Candida albicans*, the study confirms the effectiveness of these herbal ingredients in controlling it.

Pepper (rich in piperine) provides antioxidant, anti-inflammatory, and antimicrobial benefits while improving scalp circulation. Bitter gourd contains antifungal compounds that soothe the scalp and promote hair strength. Similarly moringa leaves contain vitamins A, C, E, iron, zinc, flavonoids, and antimicrobial compounds that help maintain hair health and exhibit anti-dandruff activity.

The selection of excipients was carefully considered to enhance formulation efficiency and stability. Evaluation studies of shampoos showed acceptable physical appearance, pH, solid content, viscosity, good cleansing action, better foaming stability, and absence of skin irritation. Overall, the herbal anti-dandruff shampoos were found to be effective in reducing dandruff without irritation, and better conditioning performance compared to the synthetic shampoos. Hence, we concluded that these formulations offer a safer and effective alternative for managing dandruff and preventing hair fall with potential for future commercial scale up and further comparative studies with marketed products.

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