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Development and Scientific Validation of an Ethnobotanical Herbal Shampoo from Indigenous Medicinal Plants: Physicochemical, Phytochemical, Mineral and Antimicrobial Evaluation

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Abstract: After the Covid-19, the global personal care market has witnessed a significant shift towards natural and eco-friendly products. This trend is driven by growing consumer awareness about the potential adverse effects of synthetic chemicals found in conventional hair care products. Herbal shampoos, formulated with TKS and natural ingredients, offer a promising alternative and therapeutic benefits of various botanicals. So, to explore the TKS and ethnobotanical knowledge to overcome the sculp related problems. More than 18 MPs are collected, dried, and aqueous extract of some MPs is concentrated at 40°C for 1hr followed by 80°C in next 3 to 4 hrs in prime supplement Amla and other ingredients along with fragrance herbs lemon grass and foaming agents with continuous stirring to prepare chemical and non-chemical-based shampoos. The prepared samples were sent to the Baijnath Research and Development laboratory for characterisation. The pH of samples varies from 3.977-5.43, total Ash value 0.73-1.11%, and high solubility in water 99.88% chemical-based shampoo than alcohols 10.13% and acids as 0.022%. the phytochemical tests showed that the alkaloids, flavonoids, phenols and saponins were present while steroids, tannins and anthraquinone were absent. Shampoo formulation showed a good antimicrobial activity against gram +ve less than 104 CFU/g and gram -ve bacteria less than103 CFU/g whereas the mineral studies showed that the herbal shampoo possesses a significant amount of all minerals and Pb, Cd, Hg in less than 1.00 ppm through atomic adsorption spectra. The presence of other minerals which are present in good amount are found through TLC and are essential for the normal functioning of the cleansing agent, could well mean can be taken as sculp washing agent.

Keywords: *Cleansing Agent, Preparation, Organoleptic Evaluation, Phytochemicals, Antimicrobial, Washing Agent.*

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I. INTRODUCTION

Dermatological aberrations of the cranial scalp—specifically seborrheic dermatitis, intractable pruritus, cutaneous xerosis, localized erythema, premature telogen effluvium, and early-onset alopecia—have emerged as pervasive global public health concerns [1]. Comparative chronological data indicate that contemporary cohorts experience these microbially mediated pathologies at a substantially lower developmental age than previous generations [2]. This accelerated onset is largely driven by opportunistic and pathogenic colonization by diverse bacterial, viral, fungal, and parasitic consortia [3]. Because the human scalp features a remarkably high density of sebaceous glands, its naturally elevated lipid profile—compounded by the ubiquitous application of synthetic hair cosmetics—creates an ideal microenvironment for pathogenic biofilm proliferation [4,5,28]. Consequently, mitigating scalp dysbiosis necessitates regular, methodical cleansing with specialized trophological vehicles, most notably shampoos [6]. Historically, ethnobotanical frameworks and primordial traditional medicine relied heavily on pristine plant-derived formulations to preserve hair shaft integrity and maintain scalp homeostasis [7]. These

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traditional knowledge systems (TKS) utilized complex botanical matrices entirely devoid of the synthetic materials common in modern consumer products, such as parabens, petroleum derivatives, sulfates, heavy metals, and silicones [8,9,29]. Contemporary green chemistry and dermatological sciences validate these bio-based ingredients as being inherently more biocompatible and ecologically sustainable, avoiding both the localized dermal toxicity and environmental biomagnification associated with synthetic surfactants [10]. The post-COVID-19 healthcare landscape has triggered a profound paradigm shift in consumer behaviour, marked by an unprecedented global demand for Ayurvedic and TKS-aligned personal care products [11]. Plant-derived extracts are heavily favored due to their non-toxic, mild, and biocompatible profiles [12]. Crucially, these organic formulations cleanse effectively without destabilizing the scalp's delicate, naturally acidic pH range ($5.0\text{–}5.5$) or stripping the protective hydrolipid barrier, thereby establishing a higher benchmark for formulation safety and quality [13,14,30].Spurred by these clinical and market imperatives, this investigation was designed to engineer, optimize, and standardize a novel polyherbal cleansing matrix, followed by a rigorous assessment of its physicochemical profiles and antimicrobial efficacy [15]. The specific botanical candidates selected for this study possess documented antibacterial, antifungal, antioxidant, anti-greying, and pediculicidal properties; these functionalities are deeply rooted in classical Ayurvedic literature and strongly supported by contemporary in vitro and ethnopharmacological assays [16,17]. Beyond mere pathogen eradication, these specialized herbs stimulate dermal papilla cells to encourage robust hair growth while exerting a profound anti-inflammatory, soothing effect on the follicular microenvironment [18]. Although the commercial market is inundated with diverse cleansing and shampooing products, most are formulated primarily to satisfy superficial cosmetic criteria [19]. To serve as a viable, clinically sound alternative to chemical-laden synthetic formulations, an ideal bio-based surfactant system must successfully meet an explicit matrix of performance benchmarks:

- It must thoroughly emulsify and eradicate exogenous dust, particulate pollutants, and impacted sebum crusts [20].
- It must clean the hair fibres efficiently without inducing mechanical friction or structural damage to the cuticle [21].
- It must generate a dense, stable foam volume to facilitate optimal dirt dispersion, which is vital for consumer compliance [22].
- The formulation must possess low rinsability resistance, washing away easily with water without leaving occlusive chemical residues on the scalp [23].
- It must act as a natural conditioner, leaving the hair non-dry, soft, manageable, and structurally lustrous [24].
- It must impart a subtle, pleasant aroma derived exclusively from natural volatile constituents [25].
- It must exhibit absolute epidermal and ocular biocompatibility, causing zero irritation to the skin or delicate mucous membranes [26, 27] and should effectively and completely remove the dust and excessive sebum.

II. EXPERIMENTAL

➢ *Materials*

The chemicals and reagents used for the biochemical tests and comparative chemical-based shampoo formulation and antimicrobial studies were all obtained from assured importers / suppliers of pharmaceutical grade chemicals.

Table 1 Different Ingredients Used

S. No.	Ingredients	Botanical Name	Part Used	Use	Amount
	Amla	*Phyllanthus emblica*	Fruit	Conditioning and strengthening	15%
	Methi	*T. foenumgraecum*	Fruit	Antimicrobial agent	1%
	Neem	*Azadirachta indica*	Leaves	Antimicrobial agent	5%
	Tulsi	*Ocimum sanctum*	Leaves	Antimicrobial agent	5%
	China Rose	*H. rosa-sinensis*	Flowers	Anti-hair fall	15%
	Aloe-Veera	*Aloe vera*	Stem	Moisturiser	6%
	Lamb	*B. Pilosa*	Fruit	Anti-inflammatory	2%
	Neel kanta	*D. erecta*	Flowers	Anti-microbial	5%
	Fern	*P. aquilinum*	Leaves	Anti-microbial	5%
	Dudhali	*E. Hirata*	Fruit	Anti-microbial	2%
	Mikoye	*Solanum nigrum*	Fruits	Hair growth promoter	2%
	Haldi	*Curcuma longa*	Root	Anti-oxidant and many more	5%
	Tea	*Camellia sinensis*	Leavea	Natural dye and conditioner	10%
	Soap nut	*Sapindus mukorossi*	Fruit	Foaming agent	15%
	Horse Tail	*Equisetum arvense*	Stem	Anti-infl. and foaming agent	2%
	Raan-Baash	*Y. glauca*	Leaves	Antimicrobial Cleansing agent	2%
	Lamangrass	*Cymbopogon*	Leaves	Fragrance, anti-oxidant	2%
	Rose water	*Rosa*	Petals	Anti-inflammatory solvent	SOLVENT
	Karkara	*Splinthus*	Fruit	Anti-oxidant cooling agent	…

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➤ *Preparation of the Herbal Shampoo*

- *Botanical Collection and Pre-Treatment*

Fresh medicinal plants—comprising *Aloe vera*, *Azadirachta indica* (Neem), *Camellia sinensis* (Green Tea), *Sapindus mukorossi* (Reetha), *Curcuma longa* (Turmeric), *Solanum nigrum*, *Agave americana* (Rambans), and various local herbs (*Karkara*, *Dhudhiya*, Long Grass, Lemongrass, Bracken Grass, *Neelkanthi*, and *Hibiscus*)—were harvested from the local region. The collected specimens were thoroughly washed with distilled water to remove superficial dust and debris. The parenchymatous mucilage (gel) was then mechanically extracted from the fresh *Aloe vera* leaves and set aside.

- *Preparation of the Formulation Base*

✓ Primary Extraction: Finely chopped aerial and floral parts of *Karkara*, *Dhudhiya*, Long Grass, green tea, Neem, Hibiscus, Lemongrass, Bracken Grass, and Turmeric (30 g each) were decocted in 2 L of distilled water. The mixture was thermally processed at 40°C for 2 h, followed by an elevated phase at 60°C for 3–4 h until concentrated into a thick herbal decoction.

✓ Mucilage Integration: A 100 mL aliquot of the isolated *Aloe vera* gel was blended into the concentrated decoction, and the matrix was maintained at 50°C for 1 h under continuous mechanical stirring.

✓ Secondary Blending and Clarification: After cooling to ambient temperature, 50 mL of fresh *Solanum nigrum* extract was added, and the mixture was reheated at 60°C for 1 h. Concurrently, a secondary aqueous extract was prepared by boiling *Neelkanthi*, *Sapindus mukorossi*, *Curcuma longa*, and *Agave americana* (30 g each) in 200 mL of water for 30 min. Both extracts were combined thoroughly, covered, and allowed to stand undisturbed for 24 h to facilitate natural sedimentation. The supernatant was filtered to remove residual particulates, yielding the clarified shampoo base.

▪ Note: A parallel herbal hair oil formulation was synthesized using an identical botanical composition, substituting the aqueous vehicle with a mustard oil base.

➤ *Development of Comparative Formulations*

To evaluate foaming efficiency and overall quality, two distinct shampoo variants were engineered:

- Formulation I (Semi-Synthetic): The baseline herbal extract integrated with a conventional chemical surfactant (synthetic foaming agent).
- Formulation II (100% Bio-based): A completely natural formulation using the saponin-rich extract of *Sapindus mukorossi* (Reetha) as the sole natural surfactant.

➤ *In Vivo Evaluation and Laboratory Analysis*

Preliminary *in vivo* consumer acceptability trials were conducted with volunteers who applied the formulations twice weekly over a 2–5 month period. User feedback on product performance was systematically recorded. Subsequently, both formulations were submitted for laboratory characterization to analyze and compare their physicochemical properties.

- (Sample-1 Soap nuts and Sample-2 chemical for foaming)

➤ *Evaluation of Quality and Efficacy of Samples*

- *Preliminary Evaluation:* Nature, colour, taste, and odour were determined.
- *Determination of Ash Value:* The standard technique was used to determine the samples' total ash content by following equation:

Ash Value=W1-W2W1-WX100

Here, W = weight of the empty crucible in gm, W2 = weight of the crucible holding ash in gm, and W1 = weight of the initial samples in gm.

- *Determination of pH:* Using a pH metre, the pH of each sample was measured by using solution of 1% w/v.

Other tests involved antimicrobial teste, Heavy metals analysis, Nutritional value analysis, stability testing, and self-life testing to ensure the safety and quality standards.

➤ *Preparation of Extract in Aqueous, Methanol, Ethanol and Acetone by Using Noble Method for the Testing of Phytochemicals [30-35]*

- Ellagic test for phenols: Muddy brown precipitate.
- Ferric Chloride test for flavonoids: Colour green-blue.
- Sulphuric acid test for glycosides: Brown ring.
- Wagner’s test for alkaloids: Reddish-brown precipitate.
- Molisch’ test for carbohydrates: Purple colour.
- Fehling’s test for carbohydrates: Red precipitate.
- Millon’s test for protein and amino acids: White precipitate.

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III. RESULTS AND DISCUSSION

➢ *Preliminary Organoleptic Tests*

Table 2 Preliminary Organoleptic Tests of Samples

Parameters	***Sample-1***	***Sample-2***	***Evolution Results***
Colour	Brownish Yellow	Brownish Yellow	Acceptable
Odour	Pleasant and aromatic		
Texture	Smooth and homogenous	Smooth and homogenous	
Total solid	90.16%	79.68%	
pH (1% aqueous)	3.97	5.43	
Weight per ml	1.02g/ml	0.99g/ml	
Foam height	88ml	140ml	
Total Ash value (w/w)	0.073%	0.074%	
Moisture Content	66.6%	79.0%	
Acid soluble ash	0.026%	0.026%	
Water soluble	97.34%	99.88%	
Alcohol soluble	10.13%	10.14%	
Loss of Dryness (105 °C)	7.09%	7.09%	
Reducing Sugar	55.34%	56.21%	

Fig 1 a) pH Determination b) Total Ash Value

➢ *Microbiological Testing:*

To check the growth of harmful microorganisms like bacteria, moulds, and yeast to ensure samples safety from contamination as shown in the Table 3 and Figure 2.

Table 3 Harmful Micro-Organism Colony Forming Unit Per Gram

Parameters	**Testing Methods**	**Sample-1**	**Sample-2**	**Evolution Results**
Total Aerobic Plate Count (CFU/g)	Standard Agar method	$< 10^4$	Not observed	Acceptable
Yeast and Moulds count		Not Observed		
E. coli (CFU/g)		$< 10^3$	$< 10^3$	
S. aureus (CFU/g)		$< 10^2$	< 10	

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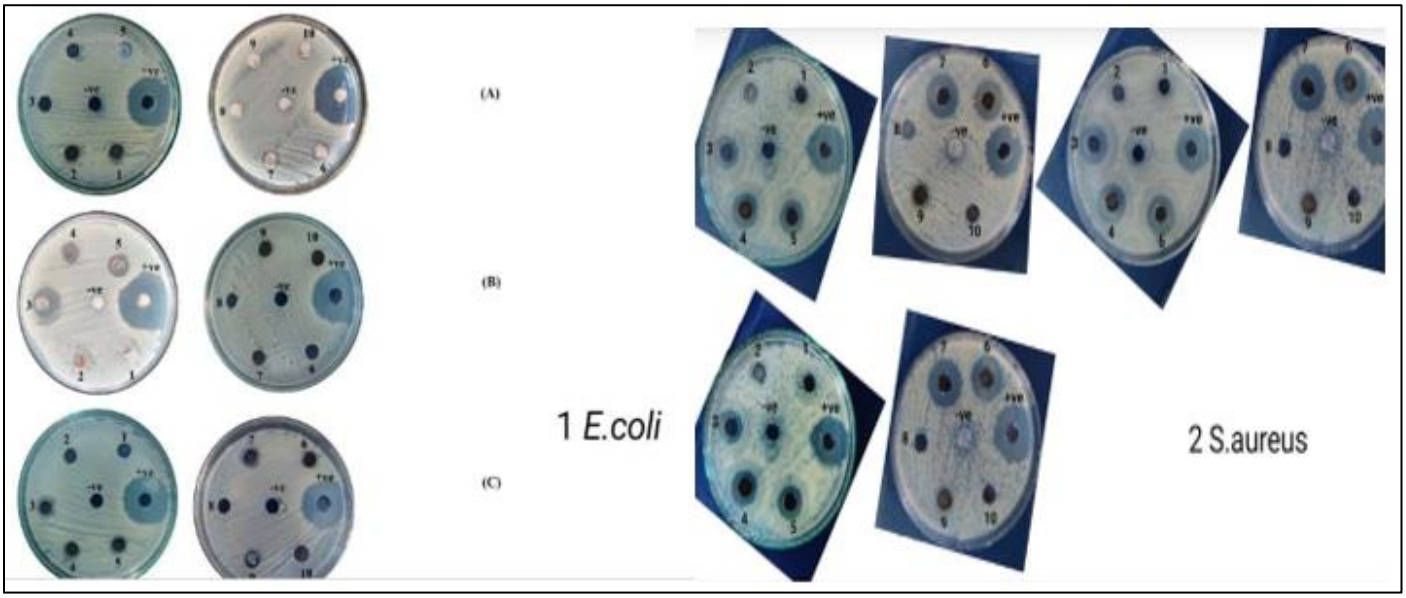

Fig 2 Shows the Antimicrobial Activities with *E. coli* and *S. aureus* Bacteria

➢ *Heavy Metal Detection*

Heavy metals like lead, mercury, and cadmium, which can be harmful if present in high proportion as shown in the Table 4.

Table 4 Different heavy metal detection in Samples

Parameters	Testing Methods	Sample-1	Sample-2 Evolution Results	
Lead (Pb) 0.72	Adsorption spectra	>1ppm	>1ppm	Acceptable
Mercury (Hg) 0.97		>0.5ppm	>0.5ppm	
Cadmium (Cd) 0.77		>1ppm	>1ppm	

➢ *Stability and self-life testing evaluation*

Samples-1 and 2 were tested for stability over time to ensure that it retains its quality, efficacy, consumption effectiveness throughout its shelf life as shown in the Table 5.

Table 5 Stability and Self-Life Tests for Samples

Parameters	Testing conditions	Sample-1	Sampl-	Evolution Results
Colour Stability	Exposure in Heat, light and moisture	No change observed		Acceptable
Odour Stability				
Texture Stability		No change	Little Change	

➢ *Average of Different Phytochemicals in Different MPs as Shown in the Table 6*

Table 6 Average Phytochemicals in Different MPs

Test of Extract	Aqueous Extract	Methanol Extract	Ethanol Extract	Acetone Extract
Alkaloids	---	+++	+++	+++
Carbohydrates	+++	+++	+++	+++
Amino acids/ proteins	---	+--	+--	+--
Glycosides	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++
Reducing Sugars	---	+++	+++	+++
Terpenoids	---	---	---	---
Anthrocyanins	---	+++	+++	+--
Carboxylic acid	+++	+++	+++	+++
Coumarins	+++	+++	+++	+++
Phenols	+++	+++	+++	+++
Quinones	---	---	---	---

➢ *Different Phytochemicals Constituents in Different MPs as Shown in the Table 7*

Table 7 Phytochemicals Constituents in Different MPs

S. No.	Phytochemical Constituent	Test Name	Principle / Reagent Used	Positive Observation	Inference
1.	Phenols	**Ellagic Test**	The extract is treated with Ellagic reagent. Phenolic compounds react to form a characteristic precipitate.	Muddy brown precipitate	Presence of phenolic compounds
2	Flavonoids	**Ferric Chloride Test**	Ferric chloride solution reacts with phenolic hydroxyl groups of flavonoids, producing a colored complex.	Green-blue colour	Presence of flavonoids
3	Glycosides	**Sulphuric Acid Test**	Concentrated sulphuric acid reacts with glycosides, producing a characteristic-coloured ring.	Brown ring at the interface	Presence of glycosides
4	Alkaloids	**Wagner's Test**	Wagner's reagent (iodine in potassium iodide) reacts with alkaloids to form a precipitate.	Reddish-brown precipitate	Presence of alkaloids
5	Carbohydrates	**Molisch's Test**	Molisch's reagent followed by concentrated sulphuric acid dehydrates carbohydrates to form furfural derivatives that react with α-naphthol.	Purple or violet ring	Presence of carbohydrates
6	Reducing Carbohydrates	**Fehling's Test**	Fehling's solution is reduced by reducing sugars to form cuprous oxide.	Brick-red precipitate	Presence of reducing carbohydrates
7	Proteins and Amino Acids	**Millon's Test**	Millon's reagent reacts with tyrosine residues in proteins to produce a precipitate or colour change.	White precipitate (may turn red upon heating)	Presence of proteins and amino acids

IV. CONCLUSION

The studies demonstrated that the quality of cleansing agent shampoo based on herbs was as per standards. It may provide safe and effective remedy for different hair problems as evidenced by its significant antimicrobial activities especially against dandruff and scalp infections. The shelf life of shampoo was found as 6-8 months under normal storage conditions. After testing the shampoo in the Baijnath Research and Development laboratory, it is found that this herbal shampoo contained high quality ingredients which are anti-dandruff, anti-itching, and antimicrobial in nature to reduced dandruff, itching and falling hair problems.

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