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Formulation Development and Evaluation of Herbal Hair Oil

Vandana Mahawar[1], Dr. Phool Singh Yaduwanshi[1], Dr. Gaurav Jain[1], Dr. Kavita R Lokesh[1]

[1]IES Institute of Pharmacy, IES University, Ratibad Road, Bhopal, Madhya Pradesh

Corresponding Author:

Vandana Mahawar

IES Institute of Pharmacy, IES University, Ratibad Road, Bhopal, Madhya Pradesh

Email ID : Vandanamahawar707@gmail.com

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ABSTRACT

Context: Plants with antioxidants and antibacterial properties are helpful in increasing blood circulation and thus good for hair growth and for the treatment of other hair related problems.

Aim: The objective of the present research work is to develop an herbal hair oil formulation by using extract of *Aloe* barbadensis, *Murray koenigii, Bacopa monnieri, Annona squamosal, Nardostachys jatamansi, Trigonella foenum-graecum and Nigella sativa* having more potency for curing hair fall.

Method: Hair oil is prepared by mixing all plant ingredients with the base oil and heated for some time to prepare herbal oil and then its evaluation is done by using parameters like physical properties, pH and viscosity, acid value, saponification value, primary irritation. Antioxidant activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method by taking ascorbic acid as standard and antimicrobial properties is determined by cup plate method by taking fluconazole (for fungi) and Ciprofloxacin (for bacteria) as standard.

Results: Herbal hair oil A4 and A5 formulation shows good antioxidant and antimicrobial activity thus good for hair growth. Both formulations are good from stability and other general parameter point of view

Keywords: Hair loss, Antioxidant, Murray koenigii, Anti-microbial activity

1. INTRODUCTION

Hair is a protein filament that grows from dermal follicles. Mammalian hair is one of their most distinguishing features. Hair is most commonly associated with hair development, hair types, and hair maintenance, but it is also an essential biomaterial made mostly of protein, particularly alpha-keratin.[1] There are several health related problems like Premature grey hair and cavities, dandruff, Baldness etc. For this human use variety of products to protect hair.[2] Hair oil are hair care products. Hair care merchandise is defined as the formulations which might be used for the reason of cleaning, editing the hair texture, providing nourishment to the hair, and retaining the healthful appearance of hair. Hair oil are hair care components implemented to the hair for the treatment of hair disorder which include baldness, greying of hair, hair fall, dry hair and also allows in providing nourishment to hair. herbal cosmetics are excessive in demand because of increasing hobby of mankind closer to them additionally natural cosmetics are more effective with negligible facet consequences and components are without problems to be had. Natural hair oil is a critical part of natural cosmetics. Natural hair oil is greater desired and used in lots of aliments of hair. They are now not most effective sell hair boom however additionally offer vital moisture to the scalp rendering in stunning hair.[3]

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Table 1: hair growth promoting herbs and its uses

Herbs	Uses
Aloe barbadensis	Act as antioxidant and provide strength to hairs, restore hair strands provide shine to hair. [4,5]
Murray koenigii	Strengthens the hair fibre, help in reducing greying of hair, useful in treating damaged hair, strengthening thin hair shaft, provide limp hair bounce, dandruff and hair fall treatment. [6,7]
Nardostachys jatamansi	Involved in prolongation of anagen phase and enlargement of follicles.[8]
Bacopa monnieri	Shows the antioxidant activity which improves the health of hair by increasing follicular size and a lengthening of the anagen phase. [9]
Trigonella foenum-graecum	Nicotinic acid present in fenugreek important for growth of hair. Lecithin provides moisture and strength to the hair thus act as a natural emollient, effective against dandruff related problems, dermatitis and dry scalp. [10]
Nigella sativa	Reducing free radical ad anti-inflammatory effects, increase hair thickness and density. [11]
Annona squamosa	Reduce free radical and help in hair restoration, improve hairs quality, prevent hair loss and promote hair progression.[12,13]

2. MATERIALS AND METHODS

Leaves, roots and seeds of *Aloe barbadensis miller, Murraya koenigii, Bacopa monnieri, Annona squamosa, Nardostachys jatamansi, Nigella sativa, Trigonella foenum-graecum was collected and authenticated.*

Preparation of Herbal Oil Formulation

Clean and dried all the glassware's to avoid contamination. Leaves, roots and seeds of *Aloe barbadensis*, *Murray koenigii, Nardostachys jatamansi*, *Annona squamosa, Bacopamonnieri, Trigonellafoenum-graecum, Nigella sativa* was washed and dried. Dried leaves, roots and seeds were crushed to coarsely powder. Hair oil is prepared by traditional method in which herbal ingredients are boiled with coconut oil, til oil and almond oil with continuous stirring. After completing boiling process filter the prepared oil through muslin cloth and transfer it in a suitable container. [14] [Table no. 2]

Table 2: Composition of the herbal oil formulations

Ingredients	F_1	F_2	F_3	F_4	F_5
Aloe barbadensis	2g	2g	2g	2g	2g
Murray koenigii	3g	3g	4g	6g	8g
Nardostachysjatamansi	2g	4g	6g	8g	9g

Bacopamonnieri	-	2g	2g	2g	2g
Trigonellafoenum-graecum	2g	3g	5g	6g	6g
Nigella sativa	2g	4g	5g	7g	9g
Annona squamosa	3g	3g	3g	4g	4g
Almond Oil	5ml	5ml	5ml	5ml	5ml
Coconut oil	125ml	125ml	125ml	125ml	125ml
Til Oil	up to 200 ml	up to 200 ml	up to 200 ml	up to 200 ml	up to 200 ml

Figure 1: Herbal hair oil formulations

Evaluation Parameters

1. Organoleptic Property

Color: Detected by naked eyes.

Odour: Detect by using sensory organ.

Grittiness: Rubbing between fingers and observed.

Sedimentation: Whole preparation is allowed to stand overnight and sedimentation is observed.

2. pH

Two ways of determining the potential of hydrogen ion concentration are

Using pH paper: Dip pH paper in a sample formulation and observed colour change.

Using pH meter: Before determining the pH of a sample pH metre is calibrated with buffer solution of pH 4 and pH 7. Place the formulation in the beaker dip the electrode in it and wait until reading appears on the meter. Note down the reading in triplicate.

4. Acid Value

About 10g of tested sample was added with 25ml of ether and 25ml of ethanol. Phenolphthalein is used as an indicator and titration was performed with 0.1M Potassium hydroxide solution. [15,16,17]

n: Number of ml 0.1M KOH

w = Wt. of oil

5. Saponification value

Take iodine flask of about 250 ml and transfer accurately weighed 2g of formulations. 25 ml of 0.5M alcoholic potassium hydroxide was added to the flask and boiled for 30 min. under reflux on a water bath. Used phenolphthalein as an indicator and titrated against 0.5M HCl('a' ml). Similarly without the sample blank was performed ('b' ml). Average of the reading was recorded.

Saponification Value: 28.05 (b-a)/w. where, w= weight in grams of the solution.

6. Specific gravity

For determining specific gravity of prepared formulations specific gravity bottle is used. Average of the reading was recorded.

7. Refractive Index

Refractometer is used to determine the refractive index. Procedure as follows:

For the measurement of refractive index instrument is placed infront of sodium light source or ordinary light source.

Focus into the eyepiece by moving upward and downward, similarly focuses the scale eye piece.

For the desired light adjust the reflector, then scale knob is rotated and see into the eye piece and reflect zero with the line. After that sample is tested in the instrument. For testing the sample specific temperature is required for that thermometer is fitted at space by screw cap, rubber tube is attach at inlet tap and hot or cold water is supplied through water bath and then reading is recorded. For placing the thin solution sample in inlet hole dropper is used and brush or glass rod is used for thick solutions. [18,19]

Determination of Anti-microbial activity by cup plate method of herbal hair oil

Cup-plate method is used to determine the antimicrobial activity of herbal hair oil formulations. Prepare culture plates by pouring 30 ml of Mueller Hinton Agar medium into sterile petridish. Media was inoculated with 0.2 ml of inoculums suspension. When liquefied inoculated medium solidify completely then with the help of cork borer wells of 4-5 mm diameter was made. Labelled the plates and add 100ul of oil solutions (at different concentration of oil solutions i.e. 100, 200, 300 and 400 µg/100 µl). Plates were incubated at 37ºc for 24 hours in case of bacteria & at 27^0c for 48 hours in case of fungi. After incubation period was over, the zone of inhibition was measured. Triplicate was performed and the experiment was repeated thrice and the average values were recorded. The herbal formulations was compared with the zone of inhibition produced by standard fluconazole (for fungi) and Ciprofloxacin (for bacteria).[20,21]

Determination of DPPH scavenging activity of herbal hair oil

Free radical scavenging activity of hair oil formulations was measured by radical scavenging ability using stable free radical DPPH. 0.1mM solution of 1,1-Diphenyl-2-picrylhydrazyl in acetone was prepared. Prepared DPPH solution of about 2ml was mixed with 3 ml of formulations solution at different concentration (50, 100, 150, 200, 250 ug/ml). Place the sample solutions in a dark place for 30 min at room temperature. After the chemical reaction occur within 30 min. absorbance was measured at 517 nm by using UV spectrophotometer.

IC 50 value means the concentration of sample required to inhibit 50% of the DPPH stable free radical. This IC 50 value of the formulation is calculated by using log dose inhibition curve. Use ascorbic acid as a positive control. If the reaction mixture shows lower absorbance then scavenging activity of formulations will be high. % RSA was determine by following formula: [22,23,24]

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of sample})}{(\text{Absorbance of Control})} \times 100$$

Determination of Stability of herbal hair oil and hair cream formulations by accelerated Stability Studies

Study observed the physical change and an increase rate of chemical degradation of herbal formulation by using exaggerated storage conditions as part of the formal stability testing programme.[25] Stability of herbal formulations was evaluated by placing formulations on different storage conditions i.e. 8, 25 and 40^0c at 75% RH for 6 months.[26]

3. RESULT AND DISCUSSION

Various herbal hair oil formulations (A1, A2, A3, A4, and A5) are prepared. Every formulation includes varying concentrations of plant powder. All the plant components are blended with the base oil and heated for a period of time to create herbal hair oil, followed by the assessment of parameters such as physical traits, pH and viscosity, acid value, saponification value, refractive index, and primary irritation. All the herbal hair oil formulations appeared greenish-brown and exhibited suitable pH, viscosity, acid value, and saponification value, refractive index, without any grittiness or sedimentation issues. Following a comprehensive assessment, all herbal hair oil formulations undergo antimicrobial and antioxidant evaluations.

Antimicrobial activity

The cup plate method is utilized to assess the antimicrobial activity of herbal hair oil formulations. Fluconazole exhibits an inhibition zone of approximately 2.06±0.052 (for Candida Albicans), while Ciprofloxacin (against Staphylococcus aureus) displays an inhibition zone of around 2.01±0.039, with both serving as standards. The herbal hair oil formulations A1, A2, A3, A4, A5 demonstrate inhibition zones of 0.79±0.028 CA/0.77±0.027 SS, 1.10±0.052 CA/1.12±0.062 SS, 1.39±0.081 CA/1.37±0.028 SS, 1.80±0.027 CA/1.79±0.071 SS, and 1.85±0.041 CA/1.83±0.050 SS, respectively. Of all the formulations, A4 and A5 exhibits the largest zone of inhibition.

Antioxidant activity

The antioxidant activity of herbal hair oil formulations was assessed using DPPH radical scavenging activity. Ascorbic acid used as standard shows % RSA about 91.19±1.98 with IC 50 of 28.75±2.33. Herbal hair oil formulations A1, A2, A3, A4, A5 shows antioxidant activity of about 58.80±0.064, 64.78±0.39, 75.96±0.68, 84.78±0.91, 88.82±0.71 respectively. Among all herbal hair oil formulations A4 and A5 herbal hair oil formulation shows maximum antioxidant activity.

Stability Studies

Herbal hair oil formulations A4 and A5 demonstrate superior antioxidant and antimicrobial activity; consequently, these formulations are subjected to a stability study using accelerated methods over a period of 6 months. Herbal formulations A4 and A5 are placed at different storage conditions and then evaluated for their physiochemical properties. Parameters like colour, odour, grittiness, sedimentation, acidity, saponification value, irritancy test, antioxidant, and antimicrobial activity of A4 and A5 formulations are evaluated. Other parameters like specific gravity and refractive index of oil formulations are also evaluated for 6 months at different time intervals: the initial month, after 3 months and after 6 months. All the physicochemical parameters were well maintained during the period of accelerated stability studies at 80°C in the refrigerator and at 25°C and 40°C in the incubator for 6 months of A4 and A5, but very slight sedimentation is observed in formulation A5.

Table 3: Evaluation Parameters of different herbal hair oil formulation

Parameters	A1	A2	A3	A4	A5
Colour	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	No sedimentation	No sedimentation	No sedimentation	No sedimentation	Very Slight sedimentation observed
Acid Value	1.57±0. 040	2.39±0.027	2.00±0.032	1.81±0.054	2.19±0.045
Saponification Value	137±4.7	144±4.1	155±2.9	134±3.6	157±2.4
Specific Gravity	0.889±0.0025	0.915±0.0021	0.935±0.0028	0.974±0.0024	0.963±0.0025
Refractive Index	1.498±0.018	1.564±0.025	1.523±0.043	1.487±0.035	1.476±0.039
pH	6.2±0.36	5.7±0.29	6.0±0.14	5.5±0.32	5.7±0.21
Viscosity	29.47±0.19	32.85±0.24	28.31±0.31	34.78±0.41	31.65±0.25
Irritancy	Nil	Nil	Nil	Nil	Nil
Antimicrobial Activity	0.79±0.028 CA/0.77±0.027 SS	1.10±0.052 CA/1.12±0.062 SS	1.39±0.081 CA/ 1.37±0.028 SS	1.80±0.027 CA/1.79±0.071 SS	1.85±0.041 CA/1.83±0.050 SS
Antioxidant Activity	58.80±0.064	64.78±0.39	75.96±0.68	84.78±0.91	88.82±0.71

Table 4: Accelerated stability study of herbal hair oil A4 formulation

Parameters	Initial Month		
	Temperature		
	4-8 ^{0}C	Room Temperature	Above 40 ^{0}C
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	No sedimentation	No sedimentation	No sedimentation
Acid Value	1.68±0.067	1..86±0.054	2.45±0.038
Saponification Value	139±2.54	136±1.47	148±2.01
Specific Gravity	0.968±0.009	0.987±0.017	0.975±0.014
Refractive Index	1.456±0.098	1.488±0.054	1.475±0.044
pH	5.8±0.37	5.6±0.64	6.1±0.18
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.78±0.89(CC)/ 1.78±0.96 (SA)	1.79±0.39(CC)/ 1.76±0.54 (SA)	1.76±0.28(CC)/ 1.77±0.66 (SA)
Antioxidant Activity	84.57±1.39	82.41±2.60	78.14±1.98
After 3 months			
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	No sedimentation	No sedimentation	No sedimentation
Acid Value	1.88±0.058	2..16±0.034	2.89±0.065
Saponification Value	142±1.96	141±2.18	162±2.75
Specific Gravity	0.979±0.045	0.980±0.028	0.989±0.065
Refractive Index	1.487±0.055	1.496±0.19	1.509±0.081
pH	5.6±0.41	5.4±0.47	5.9±0.77
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.80±0.73(CC)/ 1.76±0.99 (SA)	1.79±0.88(CC)/ 1.77±1.69 (SA)	1.74±0.53(CC)/ 1.76±1.32 (SA)
Antioxidant Activity	82.01±1.58	84.57±2.08	77.34±2.47
After 6 month			
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness

Sedimentation	No sedimentation	No sedimentation	No sedimentation
Acid Value	2.35±0.046	2..26±0.099	2.84±0.051
Saponification Value	145±1.21	143±1.68	156±2.02
Specific Gravity	0.979±0.033	0.980±0.075	0.989±0.062
Refractive Index	1.472±0.085	1.485±0.27	1.494±0.19
pH	6.0±0.98	5.7±0.62	6.2±0.31
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.75±0.92(CC)/ 1.78±1.03 (SA)	1.80±0.65(CC)/ 1.78±1.11 (SA)	1.72±0.41(CC)/ 1.74±1.64 (SA)
Antioxidant Activity	82.34±1.28	83.98±2.31	79.68±1.42

Table 5: Accelerated stability study of herbal hair oil A5 formulation

Parameters	Initial Month		
	Temperature		
	4-8 ^{0}C	Room Temperature	Above 40 ^{0}C
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	Very slight sedimentation	Very Slight sedimentation	Very Slight sedimentation
Acid Value	1.89±0.17	2..41±0.049	2.85±0.026
Saponification Value	151±2.23	158±2.44	160±1.10
Specific Gravity	0.998±0.085	1.002±0.047	0.986±0.084
Refractive Index	1.501±0.056	1.492±0.038	1.483±0.051
pH	6.2±1.03	6.0±0.85	5.8±0.70
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.78±0.89(CC)/ 1.81±1.33 (SA)	1.82±0.39(CC)/ 1.84±1.58 (SA)	1.79±0.28(CC)/ 1.77±0.52 (SA)
Antioxidant Activity	85.17±1.57	85.89±1.85	80.54±1.14
After 3 months			
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	Very Slight sedimentation	Very Slight sedimentation	Very Slight sedimentation
Acid Value	2.21±0.063	2..48±0.080	2.69±0.076

Saponification Value	156±2.96	159±1.08	161±1.15
Specific Gravity	0.980±0.078	0.972±0.040	0.983±0.050
Refractive Index	1.501±0.032	1.491±0.98	1.498±0.080
pH	6.2±0.19	5.7±0.73	6.3±0.66
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.82±1.39(CC)/ 1.80±0.65 (SA)	1.84±0.96(CC)/ 1.83±0.98 (SA)	1.78±1.60(CC)/ 1.80±0.83 (SA)
Antioxidant Activity	82.40±2.28	83.98±1.26	78.69±1.68
After 6 month			
Colour	Greenish brown	Greenish brown	Greenish brown
Odour	Characteristic	Characteristic	Characteristic
Grittiness	No grittiness	No grittiness	No grittiness
Sedimentation	Slight sedimentation	Slight sedimentation	Slight sedimentation
Acid Value	1.98±0.089	2..37±0.19	2.76±0.042
Saponification Value	155±1.33	159±2.34	168±1.25
Specific Gravity	0.977±0.054	0.981±0.074	0.985±0.038
Refractive Index	1.487±0.051	1.495±0.90	1.514±0.48
pH	6.3±0.57	5.9±0.68	6.2±0.92
Irritancy	Nil	Nil	Nil
Antimicrobial Activity	1.80±1.36 (CC)/ 1.79±1.55 (SA)	1.85±1.85(CC)/ 1.82±0.71 (SA)	1.76±0.97(CC)/ 1.72 ±1.17 (SA)
Antioxidant Activity	81.63±2.17	82.59±1.96	77.74±1.07

4. CONCLUSION

Natural products are highly in demand because they have no side effects, are effective and economical in nature. Among all prepared formulations, A4 and A5 show good antioxidants and antimicrobial activity and other parameters are within the limits. It was concluded that herbal oil is useful in preventing hair loss caused by stress, pollution, western lifestyle and various other factors and also promotes growth of new hair

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