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Formulation and physicochemical evaluation of polyherbal hair oil for prevention of hair fall

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ABSTRACT

The objective of the present investigation was to prepare herbal oil formulations for prevention of hair loss. The oil formulations were prepared in coconut oil base using extracts from the leaves of *Tinospora cordifolia*, pulp from *Tamarindus indica*, seeds of *Trigonella foenum graecum* and flowers of *Hibiscus rosa sinensis*. The results of phytochemical screening suggest that most of the while flavonoids were present in the methanolic and aqueous extracts of *Tinospora cordifolia*, they were found only in the aqueous extracts of the other plant materials. The extracts were mixed in varying proportions with coconut oil to prepare the hair oil formulations. The formulations were evaluated for physicochemical properties. The saponification value of the oil formulations was calculated to be 247 to 265 mg of KOH/ g of oil. The iodine value of the herbal hair oil was calculated to be ranging from 7.45 to 11.80 g of I/ 100 g of oil. The density of hair oil was formulations ranged from 0.898 to 0.961 mg/mL. The pH was found to be from 5.96 to 6.31. The refractive index of all the formulations ranged between 1.457 to 1.536.

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Introduction

Androgenetic Alopecia (AGA) is a nonscarring alopecia that affects both men and women. It is characterized by a progressive miniaturization of hair follicles with a characteristic pattern distribution in genetically predisposed men and women [1]. It is the most frequent type of hair loss in both sexes [2-4]. It usually appears in the third and fourth decades and affects 30% to 50% of men by the age of 50 and around 80% of caucasian men aged over 70 years. According to data by National Institute of Health, more than 30% of adults and 12% children use treatment other than conventional treatments. In the treatment of alopecia, there is an unmet need for therapies providing satisfying, long-term results. Patients often turn to complementary and alternative medicine (CAM) in an attempt to find safe, natural, and efficacious therapies to restore hair. Treatment of hair loss requires a multimodal approach and the use of CAM may provide added benefits. Vitamins and trace minerals are vital to the hair follicle cycle and maintain homeostasis as enzyme cofactors, hormones, antioxidants, and immunomodulators. India is a repository of medicinal plants. Besides health-care, herbs are also used for beautification of the body and for preparation of various cosmetics. In traditional system of medicine, many plantsand herbal formulations are reported for hair growth promotion but lack of sound scientific backing and information limits their use. *Hibiscus rosa sinensis* [5], *Tamarindus indica* [6], *Trigonella foenum-graecum* [7] and *Tinospora cordifolia* [8] have been reported to have role in prevention of hair fall or promoting growth and volume of hair. Hence it was envisioned to develop a polyherbal formulation containing the above plant extracts that would be helpful in preventing hair fall and promoting hair growth.

Material and Methods

Collection of plant material

Tinospora cordifolia (TC) leaves were collected from the botanical garden of the institute, *Tamarandus indicus* (TI) fruit was purchased from local market, *Hibiscus rosa sinensis* (HR) flower was collected from the botanical garden of the institute, and *Trigonella foenum graecum* (TFG) seeds were purchased from local market. All the plant material was authenticated by the botany department of Safia Science College, Bhopal. The plant material was made free from any debris and unwanted material, and ground to fine powder using blender at slow speed. The powdered material were stored in air tight flasks till use.

Extraction of herbal constituents

The powdered plant material was extracted successively in petroleum ether, methanol and water. Briefly, 100 g of the powdered material was filled in a paper thimble and place in the soxhlet extractor. 150 mL of solvent was poured down the thimble to the flask attached to the extractor. The solvent was heated to extract the constituents until the extraction was completed (as visible by colorless solvent in the siphon tube of the extractor). The flask was detached from the extractor and the solvent was evaporated using rotary vacuum evaporator. The resinous residue left behind was dried and stored in air tight container. The same marc was used for extraction with all the three solvents.

Phytochemical Screening [9, 10]

The plant extracts were subjected to phytochemical analysis to detect the presence of various phytoconstituents by chemical test such as Molish, fehling solutions, benedict solutions (Carbohydrate); Libermann-Buchard (Steroids); ferric chloride, Gelatin solution test(Tannins); Keller-killani test (Glycoside); wagners, dragendroff (Alkaloids); Hemolysis test (Saponine) and Xanthoprotien test (Proteins).

Test for alkaloids

Small portion of the extract was stirred with a few drops of dilute hydrochloric acid (HCl) and then filtered. The filtrate of resulting solution was then analyzed with various reagents.

Wagner's test: A few drops of Wagner's reagent were added to few ml of plant extract sample along the sides of test tube.

Dragendroff's Test: A few drops of Dragendroff's reagent were added to 1 ml of the each extract sample.

Glycosides

Saponin glycosides

Froth test: 1 ml solution of the extract in water was placed in a test tube and shaken vigourously.

Anthraquinone glycosides

Borntrager's test: The extract was boiled with 1.0 ml of dilute sulphuric acid in a test tube for 5 minand filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane and the lower layer (dichlormethane)was separated and shaken with half its volume of dilute ammonia.

Cardiac glycosides

Kedde's test*:* The extract was *e*xtracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol are added to the above residue. The solution is made alkaline with 20% sodium hydroxide solution.

Keller killiani test (Test for deoxy sugars): The extract was *e*xtracted with chloroform and evaporated to dryness. To the residue was added 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride.The solution was transferred to a test tube and 0.5 ml of conc.sulphuric acid was added along the wall of the test tube.

Tannins and phenolic compounds

Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride.

Ferric chloride test: To the extract was added a freshly prepared solution of ferric chloride.

Vanillin hydrochloride test: Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.

Alkaline reagent test: Test solution of the extract was treated with sodium hydroxide solution.

Flavonoids

Shinoda test: To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.

Zinc hydrochloride reduction test: To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.

Alkaline reagent test: To the test solution a few drops of sodium hydroxide solution was

added. Later if colour appeared, a few drops of conc. HCl were added to it.

Proteins and amino acids

***Millons test*:** Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).

Ninhydrin test: The solution of extract was boiled with 0.2% solution of ninhydrin.

Steriods and triterpenoids

Salkowski test: The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

Test for carbohydrates

Molisch Test: To the extract was added a few drops of Molisch reagent and concentrated sulfuric acid was flown down the test tube and was observed for formation of purple color.

Formulation of hair oil

The polyherbal hair oil was prepared using varying concentration of the extracts rich in flavonoids in coconut oil as the base. The weighed quantities of extracts were added to preheated coconut oil (Table 1). The contents were heated for 4-6 hours on water bath maintaining temperature between 60-70°C. The contents were cooled to room temperature and filtered using Buckner funnel with the aid of vacuum.

Evaluation of formulations

The physicochemical parameters like pH, iodine value, saponification value, viscosity, density and appearance were assessed for all the oil formulations.

Organoleptic features

Organoleptic features like color and odor were observed visually in a well lit and ventilated area.

pH determination

The pH of the formulated oils was assessed using digital pH meter (Labtronics, LT-53). The pH electrode was dipped in oil contained in a beaker and the pH reading was directly recorded from the digital display of the pH meter. The electrode was wiped with tissue paper, washed with water and again wiped with tissue paper before dipping in another oil sample.

Viscosity

The viscosity of the oil formulations was measured using Brookfield Viscometer (DV2T) using spindle # 63. The oil sample was taken in a beaker and spindle was immersed in it. The spindle was allowed to rotate for 1 min the oil and the viscosity was directly recorded from the display of the instrument.

Saponification Value

Accurately weighed 2 g of the oil was taken in a conical flask. The oil was dissolved in 25 mL of 0.5 N alcoholic potassium-hydroxide solution [11]. Then the reaction mixture was refluxed using a water condenser on a water-bath for half an hour. The resulting solution was cooled and titrated against a 0.5 N HCl solution adding 1 mL of phenolphthalein as the indicator. The number of mL of acid required was noted (a). An exactly identical blank experiment (without oil) was performed. Number of mL of hydrochloric acid required is noted (b).

The saponification value of the oil was calculated using the formula

$$Saponification\ value = \frac{(b-a)X\ 0.02805\ X\ 1000}{weight\ of\ oil\ taken}$$

Iodine Value

0.5 g of oil was weighed into iodine flask and dissolved into 10 mL of chloroform. To it was added 25 mL of iodine solution over 5 minutes using pipette, mixed well and was allowed to stand in a dark for 30 minutes with occasional shaking [12]. 10 mL of 15% KI was added and shaken nicely to ensure proper mixing. 100 mL of freshly boiled and cooled water was used to wash down free iodine on a stopper. It was titrated against 0.1N sodium thiosulfate until the yellow solution turned almost colourless. A small amount starch was added as an indicator and titrated until blue color completely disappeared. A blank titration was performed without adding the oil using the same procedure. The iodine value of the oil was calculated using the formula

Iodine Number = (B-S) x N x 12.69

where

B = volume of thiosulfate used in blank

S = volume of thiosulfate used in sample

N = normality of thiosulfate solution

Density

A stopper density bottle was filled with cold distilled water and kept in a water bath at 100°C for 30 minutes. The weight was taken after losing away any water drops on the bottle. After drying the bottle was filled with the extracted oil and the process was repeated to get the final weight. The relative density of the oil was calculated [13].

Refractive Index

The refractive index was determined using Abbe's refractometer. The prisms of the refractometer were cleaned using a soft tissue using acetone as the solvent. The temperature was adjusted to 40°C. A drop of the oil was placed on the lower prism and the prisms were closed and the oil was allowed to make a thin film between the two prisms. The refractive index was determined by adjusting the instrument to obtain the most distinct reading [13].

Results and Discussion

Plant material

Tinospora cordifolia leaves, *Tamarindus indica* fruit pulp, *Trigonella foenum graecum* seeds and *Hibiscus rosa sinensis* flowers (Figure 1-4) were extracted and used as the herbal components that have been previously reported to have a role in hair loss prevention or hair thickening. The plant material were collected from local area or local suppliers and authenticated from Saifa Science College Bhopal.

Extraction of phytoconstituents

Successive solvent extraction of pants yielded extracts and the yield and properties of the extracts are presented below (Table 2).

Phytochemical Screening of extracts

The results of phytochemical screening suggest that most of the while flavonoids were present in the methanolic and aqueous extracts of *Tinospora cordifolia*, they were found only in the aqueous extracts of the other plant materials. Previously it has been reported that natural extracts possessing flavonoids have been used in prevention of hairfall as well as promoting hair growth [54-60]. Based on these reports, the flavonoid rich extracts were used for preparation of various oil formulations for prevention of hair fall. Coconut oil was used as the oil base for the extracts.

Evaluation of herbal oils

The physicochemical property of the oil formu-

lations was evaluated as per reported procedures.

Saponification value

Saponification value is defined as the number of milligrams of KOH required to completely hydrolyse (saponify) one gram of the oil/fat. In practice a known amount of the oil or fat is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is titrated against a standard acid. It is the hydrolysis of fats or oils under basic conditions to get the glycerol and the salt of the corresponding fatty acid. Saponification is important to the industrial user for it helps to know the amount of free fatty acid that is present in a food material. The quantity of free fatty acid can be distinguished by determining the quantity of alkali that must be added to the fat or oil to make it neutral. A higher saponification value indicates a shorter chain length of the fatty acid and vice versa.

The saponification value of the oil formulations was calculated to be 247 to 265 mg of KOH/ g of oil (Table 3).

Iodine Value

The iodine value is a measure of the degree of unsaturation in an oil. It is constant for a particular oil or fat. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid. The oils contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bond exist. Hence, the measure of the iodine absorbed by an oil, gives the degree of unsaturation. Iodine value/ number is defined as the 'g' of iodine absorbed by 100g of the oil.

The iodine value of the herbal hair oil was calculated to be ranging from 7.45 to 11.80 g of I/ 100 g of oil (Table 3).

Density, pH and refractive index

The density of oil is a measure of its oxidation condition. As the oxidation of oil progresses its density increases. Hence measuring the density provides an indication about the health of oil. The density of hair oil was formulations ranged from 0.898 to 0.961 mg/mL (Table 4). The density increased with increasing TCAE and TIAE concentration.

The pH of the oil was measured in order to confirm its suitability for topical application. A slightly acidic pH value was obtained for all the formulations. The pH was found to be from 5.96 to 6.31 (Table 4). As all the formulations had pH value in the range of the pH of skin, they were suitable for topical application.

The Refractive Index (RI) of oil or fat is a mean for identification of nature of a particular oil due to the difference of saturation, conjugation, presence of hydroxyl substituted and chain length of fatty acids. RI is expressed as the ratio between the sine of the angle of incidence to the sine of the angle of refraction when a ray of light of a known wavelength passes from air into the oil. The refractive index of all the formulations ranged between 1.457 to 1.536 (Table 4). The refractive index increased with the increasing percentage of TIAE in the extracts.

Conclusion

In the present investigation herbal hair oil formulations were prepared using coconut oil base mixed with flavonoid rich extracts from four different plant materials. Previous studies have linked flavonoids to prevention of hair fall. In the next phase of the studies, the effect of the

oils on hair fall prevention, hair growth, improvement in hair thickness and other follicle regeneration parameters would be studied. The present study has revealed that all the formulations were suitable for application on scalp and would possess sufficient stability and not become rancid.

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Figure 1 (A) *Tinospora cordifolia* leaf (B) *Tamarindus indica* fruit (C) *Trigonella foenum graecum* seed (D) *Hibiscus rosa sinensis* flower

Table 1 Composition of various herbal oil preparations

S. No.	Ingredient	F1	F2	F3	F4	F5	F6
1	TCME	10%	10%	10%	10%	10%	10%
2	TCAE	5%	10%	5%	10%	5%	10%
3	TIAE	15%	15%	20%	20%	25%	25%
4	TFGAE	5%	5%	5%	5%	5%	5%
5	HRAE	10%	10%	10%	10%	10%	10%
6	Coconut Oil	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL	qs 100 mL

TCME – *Tinospora cordifolia* methanolic extract; TCAE – *Tinospora cordifolia* aqueous extract; TIAE – *Tamarindus indica* aqueous extract; TFGAE – *Trigonella foenum graecum* aqueous extract; HRAE – *Hibiscus rosa sinensis* aqueous extract; qs – quantity sufficient

Table 2 Yield and properties of the extracts

Plant material	Extract	Yield (%)	Color	Physical Appearance
Tinospora cordifolia leaves	Petroleum Ether	0.86	Dark Brown	Semisolid
	Methanol	0.93	Dark Brown	Semisolid
	Aqueous	4.5	Dark Brown	Semisolid
Tamarindus indica fruit pulp	Petroleum Ether	1.9	Dark Brown	Semisolid
	Methanol	9.6	Dark Brown	Semisolid
	Aqueous	14.4	Dark Brown	Semisolid
Trigonella foenum graecum seeds	Petroleum Ether	6.7	Dark Brown	Semisolid
	Methanol	9.8	Dark Brown	Semisolid
	Aqueous	4.4	Dark Brown	Semisolid
Hibiscus rosa sinensis flowers	Petroleum Ether	1.1	Dark Brown	Semisolid
	Methanol	8.7	Dark Brown	Semisolid
	Aqueous	13.2	Dark Brown	Semisolid

Table 3 Saponification & Iodine value of oil formulations

Formulation	Safonification Value KOH/ g of oil	Iodine Value g of I/ 100 g
F1	261	7.91
F2	253	7.45
F3	259	8.96
F4	247	10.11
F5	261	10.89
F6	257	11.80

Table 4 Density of oil formulations

Formulation	Density (mg/mL)	pH	Refractive Index
F1	0.898	6.10	1.457
F2	0.903	6.08	1.489
F3	0.911	5.96	1.496
F4	0.936	6.31	1.504
F5	0.944	5.98	1.519
F6	0.961	6.19	1.536

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