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Formulation and Evaluation of Polyherbal Gel Containing Hydro-Alcoholic Extract of Some Herbs used for Hair Growth Potential

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Abstract
Herbal extract were used for time being for preparation of gel and extracts of polyhebal have a great interest in increasing its efficacy. In the present investigation hydro-alcoholic extract of *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) was taken to formulate polyherbal hair gel and was further evaluated for its efficacy. The extract of these herbs were taken for formulation of polyherbal hair gel using Carbopol 934. The prepared formulation was evaluated and the results were presented. The results indicate that the PHHG-3 have satisfactory results when compared with other formulated PHHG.

Keywords: Polyherbal gel, Herbal Extract, Hrdro-alcoholic

Introduction
In recent years, the number of men and women suffering from hair loss and/or thinning has increased globally. Hair loss is a dermatological condition, and the search for natural products that promote hair growth is never-ending [1,2]. Hair loss, also known as alopecia, is a common patient complaint that causes both psychological and physical distress [3]. Many factors, including metabolism, hormones, genetics, and the side effects of antineoplastic and immunosuppressive medicines, have harmed hair development. According to one study, androgenic alopecia affects around half of the world's adult population [4,5]. In histological analysis, it is a dyhydrotestosterone-medicated process marked by continuous shrinking of androgen reactive hair follicles and per follicular fibrosis of follicular units [6].
A variety of herbal products have been praised for their ability to promote hair growth. A number of herbal medications are recommended for hair growth stimulation in India's traditional medical system [12], but their use is limited due to a lack of scientific backing and expertise. *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) are the plant widely used for the proper growth of hair and for healthy black and long hair in India. A number of herbal products have been acclaimed with hair growth promoting activity [7].
Polyherbal are the formulations containing two or more than two herbs are called polyherbal formulations. The popularity of polyherbal formulation is due to its high effectiveness towards a number of diseases. Traditional medicine, since ages have been an important source of potentially useful new compounds to develop chemotherapeutic agents and nature is contributing to an impressive number from which numbers of modern drugs have been isolated. [8] Thus the aim of the study was to develop a polyherbal hair gel consisting of hydro-alcoholic extract of

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Material and Methods

Extract

The dried hydro-alcoholic ethanolic extract of the part material *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) were taken after extraction for formulation of polyherbal hair gel.

Formulation of polyherbal hair gel

Carbopol 940 was dispersed in 50 ml of distilled water. It was kept aside to swell, which was further stirred to form a hair gel. Required quantity of methyl paraben was dissolved in distilled water with the aid of heat on water bath. Solution was cooled and propylene glycol was added to it. Further required quantity of hydro-alcoholic extract of *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) at different concentration was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. All the ingredients were mixed properly and with continuous stirring. Triethanolamine was added drop wise to the formulation for the adjustment of skin pH (6.8-7) and also to obtain a gel at required consistency [9-10]. The formula was mentioned in Table 1.

Table 1: Formulation of polyherbal hair gel

Ingredients	Formulation Code					
	PHG-1	PHG-2	PHG-3	PHG-4	PHG-5	PHG-6
HAETA (mg)	500	500	500	1000	1000	1000
HAEMP (mg)	500	500	500	1000	1000	1000
HAEMO (mg)	500	500	500	1000	1000	1000
HAEPG (mg)	500	500	500	1000	1000	1000
HAEEA (mg)	500	500	500	1000	1000	1000
HAEMK (mg)	500	500	500	1000	1000	1000
Carbopol 934	0.25 mg	0.50 g	0.75 mg	1 gm	1.25 gm	1.5 gm
HPMC	0.25 mg	0.50 g	0.75 mg	1 gm	1.25 gm	1.5 gm
Polyethylene glycol (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Methyl paraben (mg)	0.08	0.08	0.08	0.08	0.08	0.08
Triethanol amine (ml)	1	1	1	1	1	1
Distilled water (qs 100 ml)	100	100	100	100	100	100

Evaluation of Polyherbal hair gel [11-12]

Physical evaluation

The appearance of the formulation was observed which included clarity and transparency was determined visually.

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Determination of pH
The pH of the gel was determined using a calibrated pH meter at 4 °C. The readings were taken for an average of 3 samples.

Gelling capacity
The gelling capacity was measured by visual method. 100µl sample was placed in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 35^0 C and then visually assessing the gel formation and noting the time taken for gel formation.

Gelation temperature
The gelation temperature was determined using the test-tube-inverting method. A volume of 2 ml of the *in-situ* gel was placed in a test tube, which was then immersed in a water bath at 15 °C. The temperature of the water bath was then gradually increased, samples were examined every 2 minutes, and the gelation temperature was recorded when the gel stops fowing upon test tube inversion at 90°. The readings were taken for an average of 3 samples

Viscosity
Viscosity of sols was measured using Brookfield viscometer (model DVII, Engineering Laboratories, Middleboro, MA) spindle no 01 at 20 r.p.m. at temperature 4 °C and 37 °C. The experiment was carried out in triplicate.

Syringeability study
The ability of the prepared formulations to fow easily through a syringe of 21 gauge needle was assessed using the method employed by Maheshwari. One ml of the cold gel was filled in 21 gauge needle syringe and the ability of the gel to flow under normal handling pressure was assessed.

Extrudability
A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated.

Spreadability
Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Hundred g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation.

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Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec.

Drug content

Each formulation (1 g) was taken in a 50 mL volumetric flask and made up to volume with methanol and shaken well to dissolve the active constituents in methanol. The solution was filtered through Whatman filter paper and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with methanol. The content of active constituents was estimated spectro photometrically by using standard curve plotted at 280 nm.

***In-Vitro* release studies**

A sample of 1 ml of gel was placed into a dialysis membrane 7 cm long. Bags were then suspended in 50 ml of (ethanol: water 1:1) preheated at 37± 0.5 °C in shaking water bath at 37 °C and 25 strokes per min. At predetermined time intervals, one milliliter sample was withdrawn and replaced with an equal volume of fresh medium. The whole release media were changed and replaced with fresh media every day (24 h) during the release studies duration (up to one week). Samples were diluted and analyzed using an UV spectrophotometer for tannins concentration at λ 280 nm. The cumulative amount of drug released was calculated based on a calibration curve. All experiments were done in triplicate.

Results and Discussion

The dried hydro-alcoholic extract of *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) were used to formulate polyherbal hair gel using carbopol 934 using different concentration of extract and polymers. Six different batches were prepared and were evaluated for physical appearance, pH, gelling capacity, gelation temperature, viscosity, Syringeability study, Extrudability, Spreadability and drug content.

From the results observed it was concluded that all the prepared polyherbal hair gel has good clarity and transparency. The pH so obtained was within the limit as for most of the preparation indented to be used. The pH values of all the formulations were in the close range of neutral pH and hence it caused no skin irritation. The gelling capacity and gelation temperature were found within the limit. Polymers were included in the designed topical formulations in order to provide a prompt release of drug and to achieve as well as to maintain the drug concentration within the therapeutically effective range. As the concentration of the polymer was 0.25 to 1.25 in all gel formulations no major variation in viscosity was observed. Values of the spreadability indicated that the hair gel formulations are easily spreadable. The drug content was found to be maximum of PHHG-3. From the data obtained it was concluded that the polyherbal hair gel formulated using hydro-alcoholic extract of *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) was found to be more potent and efficacious at concentration of Carbopol 934. Further the same conclusion has been confirmed by the results of *in-vitro* drug release studies.

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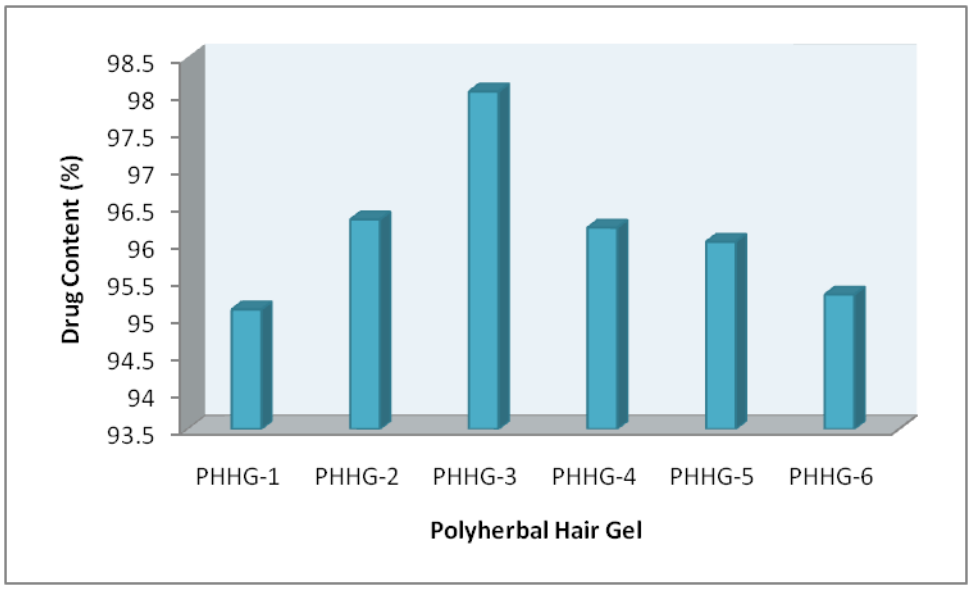
Table 2: Evaluation parameters of poly herbal hair gel

Evaluation Parameters	Formulation Code					
	PHHG-1	PHHG-2	PHHG-3	PHHG-4	PHHG-5	PHHG-6
Clarity	C	C	C	C	C	C
Transparency	T	T	T	T	T	T
pH	7.11	7.09	7.0	7.08	7.11	7.17
Gelling capacity	+	++	++	+++	++++	-
Gelation temperature	24.1	27.3	30.2	38.4	4.2	42.6
Viscosity (Poise)	0.3612	0.3721	0.3821	0.3910	0.3920	0.3822
Syringeability study	E	E	E	E	E	E
Extrudability (%)	94.11	95.21	98.19	96.09	95.10	93.21
Spreadability (gcm/sec)	58.02	61.21	73.12	68.32	67.21	58.21
Drug content (%)	95.11	96.32	98.04	96.21	96.02	95.31

Abbr: - : No gelation, + : Gel forms after some time, ++ : Gel forms immediately, +++: Immediate gelation remains for 8 hrs, ++++ : Immediate gelation remains for more than 10hrs. T : Translucent, C: Clear, E: Easily easily syringeable through 21-gauge needle at cold temperature.

Table 4: *In-vitro* drug release of poly herbal hair gel

Time (hrs)	Formulation Code					
	PHHG-1	PHHG-2	PHHG-3	PHHG-4	PHHG-5	PHHG-6
0	0	0	0	0	0	0
2	21.21	24.11	32.19	25.14	31.22	22.18
4	46.08	50.32	59.11	55.11	56.62	43.26
6	77.12	84.89	88.21	86.32	86.02	77.38
8	87.10	90.14	98.44	95.42	96.12	88.09



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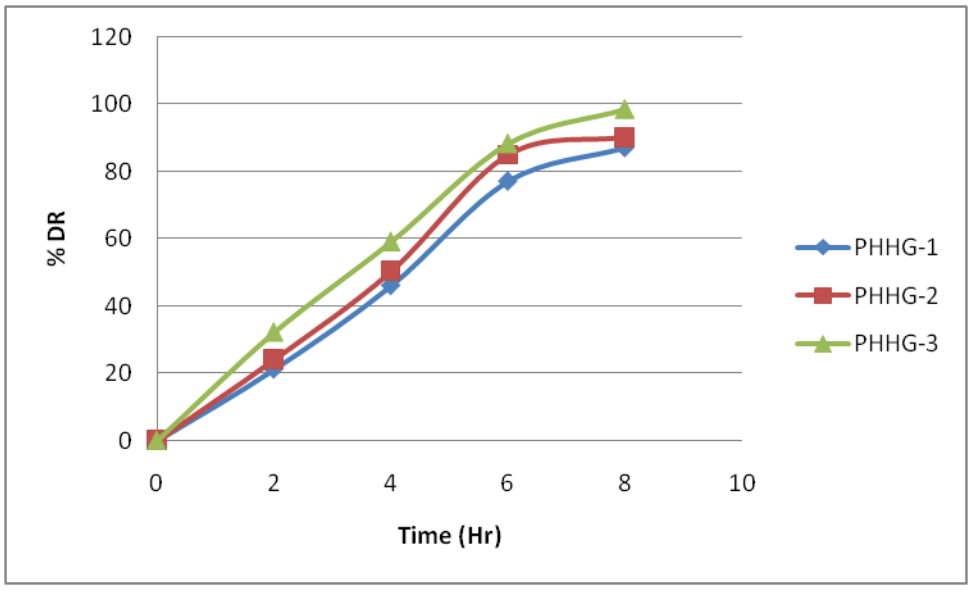
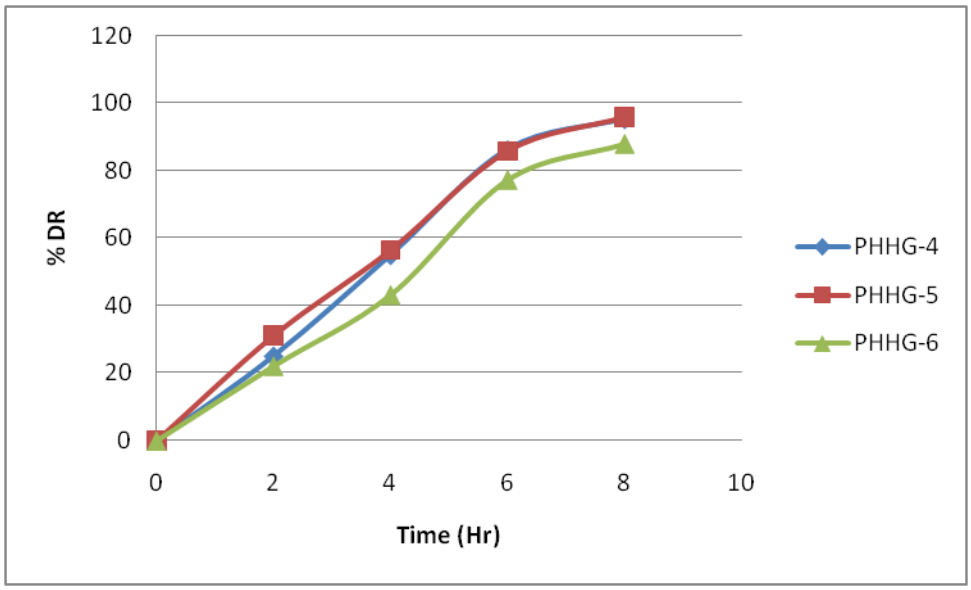


Graph 1: % Drug release of poly herbal hair gel (PHHG 1-3)



Graph 2: % Drug release of poly herbal hair gel (PHHG 4-6)

Conclusion

From the results of drug content and drug release it was found that the formulation code PHHG-3 was found to maximum therefore further this formulation was found to be suitable as compared to other tested PHHG and is considered as potent and may be investigated as biological activity is concerned to prove the efficacy of polyherbal hair gel. Hence, it was concluded from the present investigation that PHHG-3 i.e., hydro-alcoholic extract of *Triticum aestivum* Linn. (Wheat grass leaves), *Mentha piperita* Linn. (Leaves), *Moringa oleifera* Lam. (Leaves), *Punica granatum* Linn. (Fruits), *Eclipta alba* Linn. (Leaves) and *Murraya koenigii* (L.) Spr. (Leaves) is beneficial in the form of polyherbal hair gel. Moreover, further detail clinical trial may be carried in respect of its safety and efficacy profile.

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