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FORMULATION AND EVALUATION OF HAIR GROWTH PROMOTING WHEAT GRASS GEL

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ABSTRACT

Hairs are the unique feature of human body. Good quality and quantity of hairs are responsible for the self-confidence and positive attitude. Now days hair fall is one of the major problems arises worldwide. For that there are the different medications are available in market like minoxidil, finasteride, etc. But with this allopathic medication there is an observation of different side effects and due to that reason people attracted to prefer herbal medication which show minimal or no side effects. Wheatgrass is one of the richest sources of chlorophyll and it show the antioxidant activity resulting in the promotion of hair growth. In present study aqueous, alcoholic and hydroalcoholic wheatgrass extract gel was formulated and evaluated. The result of present study showed that the hydroalcoholic extract gel of wheatgrass show nearly similar effect like standard minoxidil gel with no side effects.

KEYWORD: Alopecia, cosmetics, *Triticum aestivum*, minoxidil.

INTRODUCTION

Natural cosmetics are popular one all over the world as they have better purity, safety and efficacy. Natural and eco-friendly products are becoming increasingly popular amongst the health and environmentally conscious shoppers of today. Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects with synthetic drugs. [1]

Hair is a unique character found on all mammals but not on other animals. In humans it is a special and cherished feature, especially in females, but its main functions are in protection of the skin from mechanical insults and to facilitate homeothermy; eyebrows and eyelashes, for example, stop things entering the eyes, while scalp hair prevents sunlight, cold, and physical damage to the head and neck. It also has a sensory function, increasing the perception of the skin surface for tactile stimuli, and subserves important roles in sexual and social communication, considering the psychological impact on quality of life seen in hair disorders, such as hirsutism, hair loss, etc. In particular, regarding this last point, a significantly higher prevalence of personality disorders in subjects with androgenetic alopecia regarding the prevalence of such diagnoses in the general population have been reported.[2]

Alopecia

Alopecia is a non-scarring, autoimmune hair loss on the scalp, and/or body. The etiology and pathological process of Alopecia is still unknown. The foremost common site affected is that the scalp within the kind of solitary or multiple patches of alopecia. Alopecia is a dermatological disorder that has been familiar for quite thousands of years. It is seen everywhere the world and affects close to 0.2-2% of the globe population.[3]

Triticum aestivum Linn. Commonly called wheat grass, belonging to the family: Gramineae. Triticum is a genus of annual and biennial grasses. In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear lanceolate leaves, yielding various types of wheat, native to southwest Asia and the Mediterranean region and widely cultivated almost all over the world. Generally, 15-20 species are recognized. Wheat grass is a good source of mineral nutrients. It contains significant amounts of iron, phosphorus, magnesium, manganese, copper & zinc. Wheatgrass is a rich source of tocopherols with high vitamin E potency.[4]

The presence of 70% chlorophyll, which is almost chemically identical to haemoglobin. Both chlorophyll and haemoglobin share a similar atom structure to create their respective molecules [5] The only difference is that the central element in chlorophyll is magnesium and in haemoglobin it is iron. [6]

MATERIAL & METHODS

MATERIALS

CHEMICALS & DRUGS

Minoxidil 5%w/w gel purchased from the market. Ethanol, Carbapol 934 p, PVP, Methyl paraben, Glycerine, PEG, Triethanolamine, Arachis oil, Fehlings solution A & B, Benedicts Solution, Ferric chloride, Hydrochloric acid, Sulphuric Acid, Conc. Nitric acid, Mayer's reagent, Wagner's reagent, Hager's reagent, Potassium dichromate, etc procured from the drug store of Vidyabharati College of Pharmacy, Amravati.

PLANT MATERIAL

The seeds of wheat were purchased from Bhandara District, Maharashtra, India and then it sowed in a pot, after 8th day of sowing the grass was collected and authenticated from Ms. N Kakpure, Botanist Vidyabharati Mahavidyalaya, Amravati. The wheat grass is cleaned and dried in shed. After the drying alcoholic, aqueous and hydroalcoholic extraction was carried out in the laboratory of Vidyabharati College of Pharmacy, Amravati

METHODS

Extraction procedure for wheatgrass

The powder obtained after drying wheatgrass is subjected for aqueous, alcoholic and hydroalcoholic extraction. The hydroalcoholic extraction of wheatgrass done with ethanol and water in the ratio of 30:70 respectively using Soxhlet apparatus. Whereas aqueous extract and alcoholic extraction done by using water and ethanol respectively. [7]

Phytochemical screening

Qualitative estimation of hydroalcoholic extract of Zingiber officinale were performed for the identification of various chemical constituents like alkaloid, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, glycosides and steroids. [8,9]

Formulation procedure

Herbal hair gel formulations were prepared by simple gel formulation preparation method with carbapol gel base. The gel formula contains methyl paraben, glycerine, poly ethylene glycol (PEG), carbapol 934, PVP and triethanolamine. Carbapol 934 two grams and measured quantity of extracts was dispersed in 80 ml of distilled water and mixed by stirring continuously in a magnetic stirrer at 800 rpm for 1 h. Glycerine 3 ml was added to the mixture under continuous stirring. The mixture was neutralized by drop wise addition of 50 % triethanolamine. Mixing was continued until a transparent gel was formed. The hydroalcoholic extract of ginger and wheat grass were incorporated in respective gel base and prepared herbal hair gel formulation.[10]

Table-1: Wheatgrass extract gel

Ingredients	Aqueous extract (5%)	Alcoholic extract (5%)	Hydroalcoholic extract (5%)
Carbopol 934 P	2g	2g	2g
PVP	5mg	5mg	5mg
Methyl paraben	75mg	75mg	75mg
Glycerine	3ml	3ml	3ml
PEG	6.25ml	6.25ml	6.25ml
Triethanolamine	1.5ml	1.5ml	1.5ml

Evaluation of hair gel formulation

pH: Digital pH meter was used for determination of pH of prepared wheatgras gels. For that 1 gram of gel was taken and dissolved in 100 mL distilled water and measurement of pH was done in triplicate and average value was calculated.

Viscosity: For the measurement of viscosity of the prepared gel brookfield viscometer was used. The Brookfield viscometer was rotated at 100 rpm, spindle no.64. Each reading was taken after equilibrium was attained by the sample at the end of two minutes. The study was repeated three times and average value is given in Table.

Spredability: It was determined by wooden block and glass slide apparatus. Weights of about 2 g was added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides. Spredability was then calculated by using the formula,

$S=M.L/T$

Where, S=Spredability, M=Weight tide to upper slide, L=Length of glass slide, T=Time taken to separate the slide completely from each other. The therapeutic efficacy of a formulation also depends upon its value.

Homogeneity: By visual inspection prepared wheatgrass gel was tested for homogeneity after the gel was set in the container. It was tested for appearance and presence of any aggregates.

Stability studies

The stability studies were carried out for all the gel formulations at different temperature conditions (4°, 25° and 37°) for 3 months. Known amounts of gels were taken out at different time intervals like 0.5, 1, 2 and 3 months and analysed for drug content, physical appearance and viscosity. (Sunitha 2014)

Skin irritation test

The male swiss albino mice, whose hair were removed 3 days before the experiment, were divided into 4 groups and treated as follows. The animals were treated daily up to 3 days and finally the treated skin was examined visually for erythema and oedema.[11]

Table-2: Grouping for skin irritation test

Groups	Treatment
I	Minoxidil gel 5%w/w
II	Aqueous extract gel of wheatgrass
III	Alcoholic extract gel of wheatgrass
IV	Hydroalcoholic extract gel of wheatgrass

In-Vivo Hair growth activity test

Male swiss albino mice, 18-20g, were used for hair growth studies. They were placed in cages and kept in (24°C Temp, 60%RH) standard environmental conditions, fed with standard diet and allowed free access to drinking water. The prepared formulations were assessed for the standard test.

Quantitative model developed for the study of hair growth was followed with. The mice were divided into 5 groups of 6 mice each $2cm^2$ area of dorsal portion of all the mice was shaved to remove all the hair. Where all gels were applied over the shaved area, once a day respectively as mentioned in table no.7.5. This treatment was continued for 30 days. During the course the hair growth pattern was observed qualitatively and recorded.[12]

Table 3: Grouping for in-Vivo Hair growth activity test

Groups	Treatment
I	Simple Carbopol base gel (Control)
II	Minoxidil gel 5%w/w (Std)
III	Aqueous extract gel of wheatgrass 5%w/w
IV	Alcoholic extract gel of wheatgrass 5%w/w
V	Hydroalcoholic extract gel of wheatgrass 5%w/w

Statistics

All values are expressed as mean ± SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparison Test. P value < 0.0001 were considered as significant.

RESULT

Results of Phytochemical screening of Wheatgrass extract

Treatment of the extract with different reagents like Mayer's, Wagner's reagent potassium dichromate test, lead acetate test, Shinoda test etc. it shows that the extract contains the alkaloid, flavonoid, tannins.

Table-4: - Phytochemical screening of Wheatgrass extract

Plant constituents	Test performed and reagent	Wheat grass extract
Test for carbohydrate	Molish test	+
	Benedict's test	+
	Fehling's test	+
	Barford test	+
Test for amino acid	Ninhydrin test	+
	Test for cysteine	+
Test for alkaloid	Mayer's reagent	+
	Wagner's reagent	+
	Hager's reagent	+
Test for flavonoids	Shinoda test	+
	Lead acetate test	+
Test for tannins	Potassium dichromate test	+
	Ferric chloride test	+
	Gelatine test	+
Test for protein	Biuret test	+
	Protein with sulphur	+
	Precipitation test	+
Test for phenol		+

Ash Value

Table-5: Ash Value of Wheatgrass Extract

Ash value	Alcoholic extract	Aqueous extract	Hydroalcoholic extract
Total ash	0.4%	0.5%	0.5%
Water soluble ash value	0.2%	0.3%	0.25%
Acid soluble ash value	0.1%	0.2%	0.3%

Percentage Practical Yield

Table-6: Result of percentage practical yield of obtained wheatgrass extract

Extract	Percentage Practical Yield
Alcoholic extract	16.0%
Aqueous extract	18.0%
Hydroalcoholic extract	21.0%

Physicochemical evaluation

All formulations were found to be free of grittiness, homogeneous, without phase separation with light brown colour of ginger extract gel and dark brown colour of wheat grass extract gel and combination of ginger and wheat grass extract gel viscous creamy preparation with a smooth homogeneous texture and glossy appearance.

Table-7: Physicochemical Evaluation of Formulated wheatgrass extract gels

Formulation	Homogeneity	Grittiness	Colour
Alcoholic extract gel	+++	--	Dark brown
Aqueous extract gel	+++	--	Dark brown
Hydroalcoholic extract gel	+++	--	Dark brown

Results of pH, viscosity

The pH value for the Herbal hair growth promoting gel formulations were recorded on digital pH meter shown in table and found to be in the range of 6.5 ± 0.01 to 6.9 ± 0.02. The observation revealed that all the formulations were near to neutral pH. Viscosity range of gel from 4567 to 4982 Cps was in usual range of topical gel and it was easily spreadable.[22]

Table-8: Result of pH and viscosity of prepared hair growth promoting gels of wheat grass extract

Formulation	**pH**	**Viscosity (Cps)**
Hydroalcoholic extract gel	6.9 ± 0.01	4831
Water extract gel	6.5 ± 0.01	4788
Alcoholic extract gel	6.8± 0.02	4982
Minoxidil	6.7± 0.01	4567

Result of Spredability

The finalized formulation has approximately equal spread by weight 42.41±0.12 % compared to standard Minoxidil gel 38.95±1.12%. The presence of drug in gel had not affected the Spredability of the formulation. [23]

Table-9: Result of spredability

Sr. No.	**Formulation**	**Spredability g.cm/sec**
1	Hydroalcoholic extract gel	42.42%
2	Alcoholic extract gel	40.91%
3	Water extract gel	41.16%
4	Minoxidil gel	38.95%

Results of Stability study

Stability studies of developed formulation were performed according to International Conference on Harmonization (ICH) guidelines and stability data of Hydroalcoholic formulation is shown in Table 9. It indicates that optimized formulation was stable at different temperatures (room temperature, cool temperature, elevated temperature and at 75% RH temperature) for 3 months with no separation and precipitation exhibit good stability behaviour regarding pH, appearance (no clog present), homogeneity (+++) and stable viscosity and appearance. [24]

Table 10: Result of stability studies

Temp	**Gel**	**pH**	**Viscosity**	**Appearance**
4°	Hydroalcoholic gel	+++	+++	+++
	Alcoholic gel	+++	+++	+++
	Water gel	+++	+++	+++
	Minoxidil gel	+++	+++	+++
25°	Hydroalcoholic gel	+++	+++	+++
	Alcoholic gel	+--	++-	+--
	Water gel	++-	++-	+--
	Minoxidil gel	+++	+++	+++
37°	Hydroalcoholic gel	+++	+++	+++
	Alcoholic gel	---	---	---
	Water gel	+--	+--	++-
	Minoxidil gel	+++	+++	+++

Drug content

The drug content of the gel preparations was found to be uniform among various batches prepared and was found to range from 96.40 ± 0.57 to 98.10 ± 0.32%. The drug content determination also showed that the drug was uniformly distributed throughout the gel. [22]

Table 11: Percent drug content

Sr. No.	**Formulation**	**Drug content (%)**
1	Hydroalcoholic extract gel	95.86 %
2	Water extract gel	84.32 %
3	Alcoholic extract gel	90.33 %
4	Minoxidil gel	93.92 %

In vitro drug release studies

The in-vitro drug release of the prepared gel formulations was performed using Franz-diffusion cell for 8 h and data were used to plot a graph (Figure 6.3). The drug release from all the formulations at the end of 8 hr was almost same and was ranged between 44.77±0.52 and 52.47±0.95%. It can be observed that Hydroalcoholic gel released faster rate than alcoholic, water and standard Minoxidil gels.

Formula- y= mx + c

y- Absorbance, mx+c-0.0849x + 0.0714.

Table 12: Result of percent In-vitro drug release

Sr. No	Time(hr)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
1	1	0.00	0.00	0.00	0.00
2	2	28.04	22.66	24.35	28.41
3	3	30.53	23.87	25.77	31.67
4	4	32.35	28.32	28.83	32.66
5	5	34.97	31.88	35.45	35.80
6	6	39.96	33.43	39.93	37.15
7	7	43.36	36.96	43.67	43.09
8	8	45.58	42.55	45.83	45.96
9	9	49.47	46.76	47.33	50.00

F1- Hydroalcoholic extract gel

F2- Aqueous extract gel

F3- Alcoholic extract gel

F4- Standard (Minoxidil gel)

Results of Skin irritation test

Table 13: Result of skin irritation test

Sr. No.	Formulation	Visual Observation	
		Erythema	Oedema
1	Hydroalcoholic extract gel	Nil	Nil
2	Aqueous extract gel	Nil	Nil
3	Alcoholic extract gel	Nil	Nil
4	Standard (Minoxidil gel)	Nil	Nil

Result of In vivo hair growth activity

Determination of hair length

The length of the hair began to increase until the end of the treatment course. The Hydroalcoholic wheatgrass extract gel formulation produced a nearly same effect on the length of hair when compared to minoxidil gel(standard) than other group animals' hair being 25mm at the end of the course (30th day), compared to 21mm with the alcoholic wheatgrass extract gel, 19mm with the aqueous wheatgrass extract gel and 26mm with minoxidil gel. Hydroalcoholic extract gel formulation treated groups produced a greater effect on the length of hair when compared to other groups. This may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle. This denotes, the presence of greater number of hair follicles in the anagen phase of the hair growth cycle in Hydroalcoholic group.

Table 14: Result of hair growth determination

Treatment	Mean length of hair in mm		
	10^{th} days	20^{th} days	30^{th} days
Hydroalcoholic extract gel	7 ± 0.2	18 ± 0.2	25 ± 0.2
Aqueous extract gel	4 ± 0.2	15± 0.2	19 ± 0.2
Alcoholic extract gel	6 ± 0.2	17 ± 0.2	21 ± 0.2
Minoxidil gel	8 ± 0.2	20 ± 0.2	26 ± 0.2

CONCLUSION

In this study, several hair growth gels were formulated and evaluated for their potential as an effective topical use systems for the hair growth promoting activity. The results showed that the most desirable gel formulation found to be Hydroalcoholic extract gel of wheatgrass formulation. The animal study also assured that the prepared Hydroalcoholic extract gel of wheatgrass was effective for the hair growth promoting activity without any side effect. The phytochemical and antioxidant content of medicinal plant build up the natural defences of the body, there by stimulating the organs of elimination and purification to better functioning. wheatgrass is one of such chlorophyll and antioxidant rich herb, in this study that wonder of herbal drug gives the hair growth promoting activity. wheat grass extract containing active ingredients provided more safety and compatibility which has more accessibility in pharmaceutical and cosmetic science and may promote the herbal medicine with minimal or no side effect or toxicities for hair growth therapy. Taking together all the investigation indicates that plant extract with suitable vehicle may be useful as an alternative topical medicine to minoxidil therapy Thus, it can be concluded that the herbal plant could be the promising choice for future formulations.

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