(J I D P T S)
ISSN(O):2581-6934

DEVELOPMENT AND STANDARDISATION OF POLY HERBAL FORMULATION FOR HIAR GROWTH

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Abstract: The present study was designed to formulate and evaluate polyherbal gel containing extracts of Lagenaria siceraria, Trichosanthis cucumarina, Tridax procumbent. Preliminary photochemical screening were carried out for all the plants and its extracts to determine the presence of active principle in plants.Poly herbal gel was prepared with water soluble polymer Carpool, propylene glycol400, povidone, triethanolamine to bring a good absorption capacity of the plant extracts on scalp. The standardization parameters of the gel are viscosity, pH, Homogeneity, Spread ability, content uniformity, skin irritation test all were carried out to bring a quality, purity and safety of the prepared gel formulation with the reference who applied gel without the extract. The growth of hair measured by trichoscope and the growth was completely observed after the 90 days hence, from these studies it is concluded that the prepared poly herbal gel containing Lagenaria siceraria, Trichosanthis cucumarina, and Tridax procumbens proved hair growth activity

Keywords: Carpool 934, Hair growth initiation, Hair growth completion, Histopathology, Hair follicles

Introduction

Recently, the number of men and women who suffered from hair loss and/or hair thinning is increasing in worldwide. Hair loss is a dermatological disorder, and the surge for discovering natural products with hair growth promoting potential is continuous [1,2]. Hair loss or alopecia is a common patient complaint and a source of significant psychological and physical distress [3]. Many factors such as metabolism, hormones, heredity and side effects of antineoplastic and immunosuppressant drugs, have been negatively affecting the healthy growth of hair. According to one survey, androgenic alopecia on its own eventually affects approximately 50% of the world's adult population [4,5]. In androgenic alopecia it is assumed that the genetically predisposed hair follicles are the target for androgen - stimulated hair follicle miniaturization, leading to gradual replacement of large, pigmented hairs (terminal hairs) by barely visible, depigmented hairs (vellus hairs) in affected areas [4]. It is dyhydrotestosterone medicated process, characterized by continuous miniaturization of androgen reactive hair follicles and accompanied by per follicular fibrosis of follicular units in histological examination [6].

Androgenic alopecia results in a decrease in hair follicle size accompanied by a decrease in the duration of anagen; anagen is the active phase of the hair in which hair is living and growing and an increase in the percentage hair follicles in telogen; telogen is the resting phase and accounts for 10-15% of all hairs [7]. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors [8]. Thus it is very important to develop new therapeutic materials to stop hair loss and to enhance hair growth. Alternative medicine is one interesting area, which is getting more popular. Although it has not yet been incorporated into the mainstream of medical care because of limited scientific evidences and lack of mechanistic understanding, alternative medicine is

becoming an increasingly attractive approach all over the world [9].

Herbs combine to gather one formulation it gives better curative and therapeutic effect compare when being as a single drug. Plants not directly used as medicinal purpose, when it processed and formulated as any one of the suitable formulation gives better therapeutic effect by means of dried powder form or extract from the plant with the advance technique. Gel formulation is a one of the topical formulation and it gives better absorption on the skin and less adverse effect comparable other formulation. When the plant formulated as gel it gives better absorption through skin and gives maximum therapeutic.

Lagenaria siceraria is a small shrub under cucurbitaceous family. It is grow over the tropical and subtropical area and easily available. *Lagenaria siceraria* has various ethno botanical applications and medicinal claims. It fruits have been use vegetables and the ethanomedicinal uses of leaves shows hair growth activity *Trichosanthis cucumarina*, is a small shrub under cucurbitaceous family. It is grow over the tropical and subtropical area and easily available. Its ethanomedicinal uses shows action on hair growth.

Tridax procumbent is a small weeds procumbent herb, Asteraceae family and easily available through the tropical and subtropical areas. Its leaves ethanomedical uses claim to have hair restoring properties. Among topical formulation, the gel formulation is more suitable for topical application and produce cooling effects.

PREPARATION OF EXTRACT

Extraction is the preliminary step involved in the photochemical studies. Based on solvent's polarity metabolites are extracted and according to the solubility of the constituents in the solvent. The method of extraction is hot percolation method.

METHODOLOGY

LIST OF MATERIALS AND THEIR USES IN FORMULATION

The materials used in the study were as follows:

Table 1 List of materials and their uses in formulation

S.no	Name of the Materials	Use in formulation
1.	*Legenaria siceraria* (Leaves)	Active Ingredient
2.	*Trichosanthes cucumerina*(Leaves)	Active Ingredient
3.	*Tridax procumbens* (Leaves)	Active Ingredient
4.	Carbopol 934	Gelling agent
5.	Povidone	Suspending agent
6.	Polyethylene Glycol	Moisturizing agent
7.	Tuber rose oil	Flavoring agent
.	Demineralized Water	Solvent

HOT PERCOLATION METHOD

About 200g of coarsely powdered plant was extracted with solvents of increasing polarity like Hexane, Chloroform, Ethyl acetate and Ethanol at 60-70°C. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, color and consistency of all the extracts were noted and were taken up for further

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Qualitative analysis for various phytoconstituents in the dried powders and extracts all the raw materials were carried out using different reagents are mentioned below.

DEVELOPMENT AND STANDARDISATION OF POLY HERBAL FORMULATION

The prepared extracts were taken for the preparation of topical gel with water soluble gelling agent Carpool 934, Propylene glycol 400, Providence, Glycerin, Distilled water.

METHOD FOR PREPARATION OF GEL CONTAINING EXTRACTS (1% w/w)

1 g of Carbopol 934 was dispersed in 50 ml of hot distilled water with continuous stirring. 5ml of distilled water was taken and required quantity of providence was dissolved. Cool the solution, then to that added Propylene glycol 400. Further required quantity of prepared leaves extracts were mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and tri-ethanolamine was added drop wise to the formulation for

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adjustment of required skin pH (6.8-7) and to obtain the gel at required

Consistency.

TABLE .2 FORMULAS FOR GEL PREPARATION

Ingredients	Quantity Specified
Carbopol-934	1 g
Propylene glycol-400	Quantity sufficients
Povidone	5%
Triethanolamine	1.2ml(quantity sufficient)
Distilled water	Make up to 100

Standardization of Topical Gel Formulation

- **Physical Evaluation**

  Physical parameter (50) s such as color and appearance were checked.

- **MEASUREMENT OF PH**

  PH of the gel was measured by using pH meter.

- **SPREADABILITY**

  Spread ability of the prepared gel was determined by wooden block and glass slide apparatus.

Apparatus consists of a wooden block, which was provided by a pulley at one end. Spread ability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then placed between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spread ability.

Spread ability was calculated using the following formula:

$S = M \times L / T$

Where, S = Spread ability,

M = Weight in the pan (tied to the upper slide), L = Length Moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

VISCOSITY

Viscosity of gel was measured by using Brookfield viscometer with spindle.

HOMOGENEITY

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

SKIN IRRITATION TEST

Test for irritation was performed on human volunteers. The prepared gel was applied on area of 2 square inch to the back of hand to volunteers who are selected for the evaluation

CLINICAL EVALUATION OF HAIR GROWTH,

The prepared and standardized gel formulation was evaluated clinically. The clinical evaluation carried out in the Department of Dermatology, Government General Hospital, Hyderabad.

Study groups and duration of study

30 volunteers selected according to the inclusion criteria. They are to be divided into 2 groups control and test. The control group receives gel without extract medication. The test group receives the formulated herbal gel. Duration of the study is 90days.

Application of gel

The formulated gel is applied over the scalp during the course of study. If there is any sign of irritation or discomfort to the patient, the gel is discontinued.

EVALUATION PARAMETERS FOR HAIR GROWTH (DURING THE PERIOD OF STUDY)

- Measurement of hair texture, examined by simple method of touching the patient's hair,
- Hair density/cm.sq area :examined at a fixed site of scalp with Trichoscope
- Hair loss: counted after constant combing of patient's hair for one minute with the comb

RESULTS AND DISCUSSION

PREPARATION OF EXTRACTS

The shade dried all plants materials raw were extracted in soxhlet extractor with the universal solvent ethanol. All the extracts were concentrated using rotary vacuum evaporator. The percentage yield was calculated for every extract in terms of dried weight of plant materials. The color and consistency of the

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concentrated extracts are given in table no.3 of hair growth gel. The volunteers were observed for lesions or irritation.

TABLE 3. PERCENTAGE YIELD OF EXTRACTS

S.NO	PLANT NAME	SOLVENT	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	*Legenaria siceraria* (Leaves)	Ethanol (95%)	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Dark green	5.58
2.	*Trichosanthes cucumerina* (Leaves)			Semisolid	Dark green	4.76
3.	*Tridax procumbens* (Leaves)			Semisolid	Dark green	6.46

QUALITATIVE ESTIMATION OF PHYTO CONSTITUENTS

The raw material powders and all the extracts were subjected to qualitative photochemical analysis to identify the various phytoconstituents present in it, as per the standard procedures. The results are given in the Table 8.8

FLUORESCENCE ANALYSIS

Fluorescence analysis for the extracts and the powdered drug were carried out with various reagents to identify the presence of chromospheres. The importance of fluorescence analysis is that UV light shows the fluorescent nature of the compound whereas fluorescence cannot be observed in day light. Hence it is performed according to the standard procedures. No fluorescence was observed for the powder as well as extracts indicating the absence of chromospheres in the plant.

TABLE 4. PRELIMINARY PHYTOCHEMICAL ANALYSIS OF POWDER AND EXTRACTS OF RAW MATERIALS

Chemical constituents	*Lagenarria siceraria*		*Trichosanthes cucumerina*		*Tridax procumbens*	
	Powder	Extract	Powder	Extract	Powder	Extract
Steroids	+	+	+	+	-	-
Glycosides	-	-	-	-	+	+
Saponins	-	-	-	-	-	
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Proteins	+	+	+	+	-	-
Alkaloids	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Fats and oils	-	-	+	+	-	-

+ indicates presence, - indicates absence

QUANTITATIVE ESTIMATION

TOTAL FLAVONOID CONTENT

The determination of total flavonoids from various extracts of *Lagenaria siceraria, Trichosanthes cucumerina, tridax procumbens* were performed with the Quercetin standard. The accuracy of test made by the serial dilution of Standard and the absorbance was

Measured spectrophotometrically at 435 nm. The obtained data were plotted as a Standard Calibration curve (Fig.1).

STANDARD CALIBRATION CURVE FOR QUERCETIN

To determine the accuracy of the falconoid compound by plotted the standard absorbance obtained from spectrophotometrically. The calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) of quercetin Standard stock solution, the absorbance plotted against concentration

TABLE 5.: SPECTROPHOTOMETRIC ABSORBANCE OF STANDARD AND SAMPLE

S.No	Concentration of standard solution(µg/ml)	Absorbance(435nm)
1.	20	0.135
2.	40	0.175
3.	60	0.213
4.	80	0.306
5.	100	0.347
6.	*Lagenaria siceraria*	0.178
7.	*Trichosanthis cucumarina*	0.165

Table 6 . Percentage yield of total flavonoid

S.NO	Sample	Concentration Obtained (mg/gm)	Percentage of Flavonoids Present
1.	*Lagenaria siceraria*	43.43	4.3
2.	*Trichosanthis cucumarina*	39.85	4
3.	*Tridax procumbens*	33.72	3.4

TOTAL PHENOLIC CONTENT

Total phenol content of the individual extract was determined and compared with that of standard. It is shown in table 7

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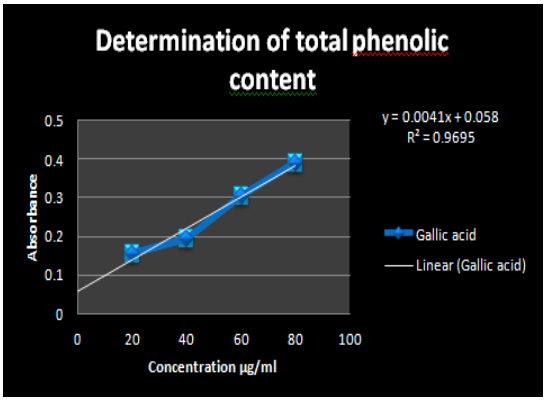
TABLE 7 SPECTROPHOTOMETRIC ABSORBANCE OF STANDARD AND SAMPLE

S.No	Concentration of Standard Solution(µg/ml)	Absorbance(435nm)
1.	20	0.156
2.	40	0.194
3.	60	0.304
4.	80	0.390
5.	*Lagenaria siceraria*	0.266
6.	*Trichosanthis cucumarina*	0.231
7.	*Tridax procumbens*	0.216

Standard Calibration Curve for Gallic acid

To determine the accuracy of the phenolic compound by plotted the standard absorbance obtained from spectrophotometrically. The calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80mcg) of Gallic acid Standard stock solution, the absorbance plotted against concentration

FIGURE .1: STANDARD CALIBRATION CURVE FOR GALLIC ACID



From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of phenolic content present in 1gm of the extract was calculated by applying the dilution factor. The concentration of each extract obtained (Table 8)

TABLE 8. PERCENTAGE OF PHENOLIC CONTENT PRESENT IN EACH EXTRACT

S.NO	Sample	Concentration Obtained (mg/gm)	Percentage of phenolic content Present(%w/w)
1.	*Lagenaria siceraria*	204.33	20.4
2.	*Trichosanthis cucumarina*	168.27	16.8

Table 9. STANDARDIZATION OF PREPARED POLY HERBAL GEL

S. No.	Parameters	Results
1.	**Physical appearance**	Light green
2.	**pH**	7.1
3.	**Spreadability**	Good
4.	**Viscosity**	43560 cps
5.	**Homogeneity**	Excellent
6.	**Skin irritation test**	No irritation

CLINICAL EVALUATION OF HAIR GROWTH

The prepared and standardized ploy herbal gel formulation was tested on human volunteers. Before the clinical evaluation was carried out ethical clearance was approved by Institutional Ethical Committee. The hair length and diameter was measured by trichoscope and the results were tabulated. Every testing interval the patients scalp photos taken with the trichoscope (Fig 3, Fig. 4).The number of hair present in scalp was counter as per square area. The obtained mean value from hair count in 1 cm square area for test group was plotted on graph

Figure .3 Hair in Scalp

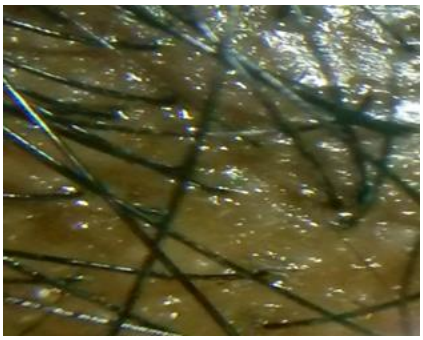


Figure .4 Hair in Scalp

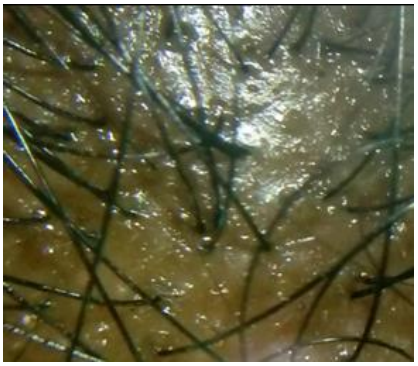


Fig..5 Mean No. of Hair count

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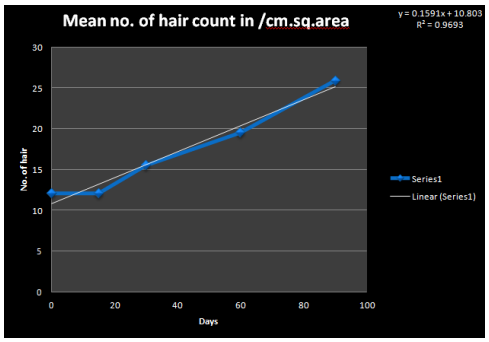
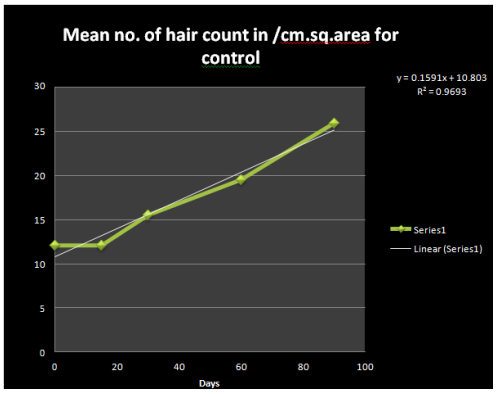


Mean No. of hair count in/cm. sq. area for Test group

Fig .6 Mean No. of hair count For Control Group



The hair growth activity of the prepared gel formulation was tested on human volunteers and growth activity was compared with the control group. The obtained mean value (Table 10) from both test and standard group were compared Fig

Table 10 Meant value of hair count

No. of Days	Test Group	Control Group
0	12.07±2.6	11.4±2.3
15	12.07±2.8	11.3±2.03
30	15.5±2.6	12.2±2.3
60	19.5±2.8	13.2±2.4
90	25.9±5.0	13.3±2.4

Values are expressed as Mean ± SD, n=15

FIG. 7 COMPARISON OF HAIR GROWTH OF TEST WITH CONTROL

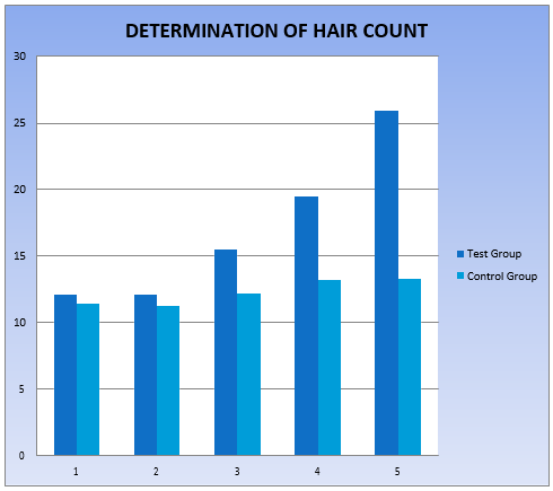


Table 11.Mean number of hair loss in Combing test

Parameter	Group	Initial 0 days	After 15 days	After 30 days	After 60 days	After 90 days
Mean hair loss (/combing)	Test	114.5± 6.4	80.4± 12.4	78.2± 11.4	69.3± 10.4	51.5± 15.4
	Control	113.2± 6.4	109.2± 6.4	103.4± 6.7	99.9± 7.9	95.2± 6.8
Perceptible Reduction in hairloss by Patients(%)	Test	-	34.1%	38.6%	45.2%	63.1%
	Control	-	4.0%	10.4%	13.7%	18.0%

Table 12 .Measurement of density

Group	Initial Mean density /cm.sq area (mm)	Mean density /cm.sq area After 15 days (mm)	Mean density /cm.sq area After 30 days (mm)	Mean density /cm.sq area After 60 days (mm)	Mean density /cm.sq area After 90days (mm)
Test	0.09±0.16	0.14±0.14	0.13±0.17	0.14±0.16	0.14±0.2
Control	0.08±0.17	0.07±0.16	0.08±0.18	0.11±0.10	0.11±0.14

CONCLUSION

Preliminary phytochemical screening were carried out for all the Lagenaria siceraria, Trichosanthis cucumarina, Tridax procumbens and their extracts to determine the presence of active principle in plants Qualitative estimation of total flavonoid contend and total Phenolic content were determined by spectrophotometrically all the extract showed significant amount of falconoid and phenolic

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compounds. Poly herbal gel was prepared with water soluble polymer Carbopol, propylene glycol 400, povidone, triethanolamine to bring a good absorption capacity of the plant extracts on scalp. The standardization parameters of the gel are viscosity, pH, Homogeneity, Spread ability, content uniformity, skin irritation test all were carried out to bring a quality, purity and safety of the prepared gel formulation The prepared poly herbal formulation was taken for the determination of hair growth activity of the selected plants The clinical evaluation of prepared gel was carried on the human volunteers and compared with the reference who applied gel without the extract. The growth of hair measured by trichoscope and the growth was completely observed after the 90 days hence, from these studies it is concluded that the prepared poly herbal gel containing *Lagenaria siceraria, Trichosanthis cucumarina, and Tridax procumbens* proved hair growth activity

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