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FORMULATION AND EVALUATION OF *ROSEMARINUS OFFICINALIS* L ON HAIR GROWTH PROMOTION IN RATS

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ABSTRACT

Hair loss or Alopecia is a dermatological disorder with psychosocial implications on patients with hair loss. Leaves of *Rosemarinus officinalis* L (Lamiaceae) is traditionally known for hair growth activity. However efficacy of *Rosemarinus officinalis* is not reported for hair growth promoting activity. We have investigated the efficacy of extract of *Rosemarinus officinalis* L on hair growth promoting properties. Hydro-alcoholic extract of *Rosemarinus officinalis* L (5%) was evaluated for qualitative, quantitative chemical test and incorporated into hydro-gel base. The formulated gel was applied topically on shaved skin of Wistar albino rats and screened for hair growth activity. Rosemary gel significantly improved hair length and hair density. The present study reveals that *Rosmarinus officinalis* L is a good hair growth promoting herb and its extract is suitable for formulation as a gel for hair growth.

KEYWORDS

Rosmarinus officinalis L, Herbal gel, Evaluation and Hair growth promotion.

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INTRODUCTON

Hair is an important cosmetic asset. Hair loss is one of the dermatological disorders in humans which is common throughout the world and is of great concern. Many factors such as metabolism, hormones, heredity and side effects of anti-neoplastic and immunosuppressant drugs negatively affect the healthy growth of hair. FDA approved drugs for hair growth are abbreviated due to their side effects. Hence there is a need for search from natural products of plant origin for agents possessing potential hair growth activity.

Various factors contribute to hair fall. Genetic predisposition and hormonal factors predominantly contribute to hair fall. Besides various disease state

such as typhoid, malaria and jaundice also cause hair fall although temporarily. The use of chemotherapeutic agents also cause hair fall. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors.

Rosmarinus officinalis L also known as rosemary (Fam. Lamiaceae) is a herb native to the Mediterranean region, but it now cultivated all over the world[1]. *Rosmarinus officinalis* L is mainly used as carminative, stimulant, diuretic, aperient, astringent, cholagogue, digestive, diuretic, emmenagogue and hypertensive[2,3]. The plant has been investigated for antimicrobial, antioxidant, insecticidal, hepatoprotective, anti-tumorogenic, Anti-inflammatory, Antinociceptive activities[4-20]. Extracts of *R.officinalis* L, contain flavonoids, phenols, volatile oil and terpenoids. Carnosol and carnosic acid are constituents of deodorized rosemary extract[21-23].

In the present study we have evaluated leaves of *Rosmarinus officinalis* L on hair growth stimulation; after formulating as a gel. Mainly two synthetic approved drugs are present for alopecia. Minoxidil, Finasteride. These two drugs are used for hair growth stimulation activity[24].

MATERIAL AND METHODS

Collection and authentication of samples

Dried leaves of *Rosmarinus officinalis* L. was used for the study. Rosemary was collected from Amruth Kesari. The sample drug has been identified and Authenticated by Dr. Shiddamalaya N, NADRI (National Ayurveda Dietetics Research Institute) Bangalore. The drug was air dried in shade and stored in polythene bags.

Preparation of extract by soxhlation

The leaves of *Rosmarinus officinalis* L. was ground using mixer grinder and powdered to mesh size of # 20. The powdered drug was then extracted by soxhlation. The prepared extract used for phyto-chemical study, formulation and evaluation.

Soxhlation method

Procedure

Soxhlet extraction was performed using classical soxhlet extraction apparatus. 10g of the sample to be extracted was placed in a thimble and 40ml of 70% alcohol was taken in round bottom flask attached to thimble. Condenser was placed above the thimble. Extraction was performed for 8hrs with 70% alcohol. Finally extract was evaporated to dryness using vacuum or rotary evaporator. The residues were weighed and the percentage yields was calculated.

Phytochemical analysis

Qualitative Chemical tests

The hydro alcoholic extract of *Rosemarinus officinalis* L was subjected to various chemical tests to detect the chemical constituents present in the drug like alkaloids, glycosides, saponins, phenols, proteins, tannins fixed oils and resins.

Quantification of phytochemical constituents

Qualitataive chemical test of extract of *Rosemarinus officinalis* L showed the presence of flavonoids, polyphenols and triterpenoids and sterols as the chief constituents. Results of the chemical constituents of the extracts are tabulated in Table No.1. The extract obtained by soxhlation method were evaluated for content of different phyto-chemicals like flavonoids, polyphenols and sterols[25,26]. The standard calibration curve is shown in Graph No.1-3, recorded in Table No.2.

FORMULATION OF *ROSMARINUS OFFICINALIS* L (ROSEMARY) GEL

The hydro-alcoholic extract of Rosemary contained more polar constituents, thus a formulation of the extract as a hydrogel was decided. In order to overcome any effects of perfume, colouring agent, anti-oxidant and preservative on hair growth. The hydro gel preparations formulated did not employ any colourant, perfume, anti-oxidant or preservative.

The hydro alcoholic extracts of *R. officinalis* L was used for preparation of gel. Carbopol 934 was used as polymer; 5% of the extract was used for gel preparation. The *Rosemarinus officinalis* L 5% hydrogel formulation was prepared.

Preparation of gel

The hydro alcoholic extract of *Rosmarinus officinalis* L (5%) was used for the preparation of herbal gel for evaluation of hair growth stimulation activity.

One gram of Carbopol 934 was dispersed in 50ml of distilled water with continuous stirring and kept overnight to get a smooth gel. 2ml of distilled water

was taken in a separate beaker and to this polyethylene glycol (PEG) 400 was added. Then 5% of the extract of rosemary was mixed in the above mixture and its volume was increased to 100ml by adding distilled water. Finally, full mixed ingredients were mixed properly with the carbopol gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjusting pH to scalp pH (pH 5.5-6) and to obtain required consistency. The same method was followed for the preparation of control gel without adding extract. However no color or perfume was included in the formulation[27].

EVALUATION OF FORMULATED ROSEMARY GEL FOR DERMAL IRRITATION AND HAIR GROWTH PROMOTING ACTIVITY IN WISTAR ALBINO RATS[28-34]

Various investigations and treatises in traditional medicine report rosemary for hair growth promoting activity. Rosemary has been incorporated into a hair cream and studied for its anti-dandruff properties clinically.

Formulation of traditional and modern of *Rosemarinus officinalis* L have been prepared and tested/used in pre-clinical and clinical models of various conditions including that for treatment of hair growth promoting activity. However there are no reports regarding evaluation of hair growth promoting activity of Rosemary. Hence the drug was evaluated for hair growth promoting activity in Wistar albino rats.

Rosemarinus officinalis Lgel (5%) was investigated for their dermal irritation and hair growth promoting activity using Wistar albino rats. The hair growth promoting activity is determined by hair length determination and hair follicle count.

Dermal irritation study

Experimental Procedure

Wistar albino rats of either sex weighing between 200-250g were divided in to four groups of six animals each. Hair from a 3cm^2 area at the dorsal portion of all the rats were sheared by applied with marketed hair remover to completely remove hair. Then the skin of rats was cleaned with surgical spirit then the formulation/control gels were applied.

Grouping of animals was done as shown below:

Group I – Control (Base) treated group

Group II – Applied with 5% Rosemary gel

All the gels (0.5gm) were applied once in a day to the shaved area.0.5gms of the herbal gel was used as the test substance was applied to an area of approximately 6cm2 of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period, i.e, 1 hour, residual test substance was removed, without altering the existing response or integrity of the epidermis. Observations were recorded after removal of the patch. Control animals were prepared in the same manner and 0.5gms of the gel base i.e., gel formulated using all ingredients except the herbal mixture was applied to the control animals and observations were made similar to the test animals. The results of skin irritation test is shown the Table No.3.

The gel was applied to the skin once a day for 7 days and observed for any sensivity and the reaction if any was graded as: A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Evaluation of hair growth promoting activity of Rosemary gel

Wistar albino rats of either sex weighing between 200-250g were divided in to five groups of six animals each. Hair from a 3cm^2 area at the dorsal portion of all the rats were sheared by applied with marketed hair remover to completely remove hair. Then the skin of rats was cleaned with surgical spirit then the formulation/control gels were applied. Minoxidil solution (2%) was used as standard for comparison.

Grouping of animals was done as shown below:

Group I – Control (Base) treated group

Group II – Applied with 5% Rosemary gel

Group V –Applied with 2% minoxidil solution

All the gels (1gm) and standard drugs (1ml) were applied once in a day to the shaved area. The treatment was continued for 30 days and hair growth pattern was observed and tabulated.

Hair length determination

Hair was plucked randomly using sterile forceps from the shaved dorsal area of rats on 15th, 20th, 25th and 30th day of the treatment. Hair length was measured and the results were recorded as mean length ± SEM of 25 hairs. The results of hair lengths are summarized in Table No.4. The results of hair length determination graph showing in Graph No.4.

Hair density

A hole of 1c.m. 2 was made on card board. Then the card board set on the desired depilated area on the back of rat after 30 days of depilation. The hair was trimmed of desired depilated area and the hair was cut with the scissors. The hair was counted manually. The results of hair density are summarised in Table No.5. Graphical depiction of hair density is shown in Graph No.5.

Statistical analysis

Data were expressed as the mean ± standard error of mean (SEM) and statistical analysis was performed using ANOVA test.

RESULTS AND DISCUSSION

Collection and authentication of samples

Dried leaves of *Rosmarinus officinalis* L used for the study was collected from Amruth Kesari. The sample drug was identified and Authenticated by Dr. Shiddamalaya N, Ref no: Drug Authentication/ SMPU/ NADRI/ BNG/ 2011-2012/ 681 Bangalore, Karnataka.

Preparation of the extract by soxhletion method

The soxhlet extract of *Rosmarinus officinalis* evaporated using rotary evaporator and the % yield calculated as 7.12%.

Phytochemical analysis

Qualitative chemical test

Extract prepared and subjected to qualitative chemical tests in order to find out chemical constituents present. The aqueous alcoholic extract of *R. Officinalis* prepared by soxhlet extraction method showed the presence of Phenols, saponins, fixed oils, 127 lavanoid 127, terpenes and di and triterpenes.

Determination of Phytoconstituents in the extract

The total phenol content, total 127 lavanoid content and Total sterol content inhydro alcoholic extract of *Rosemarinus Officinalis* L was determined by colorimetric method previously reported.

Quantitative chemical test

Standard graph for standard flavonoid, polyphenolic and sterol content

Formulation of *Rosemarinus officinalis* l (rosemary) gel

Preparation of Gels

The gel formulations are prepared according to the procedure mentioned in the methodology.

EVALUATION OF FORMULATED GELS OF ROSEMARY FOR DERMAL IRRITATION AND HAIR GROWTH PROMOTING ACTIVITY IN WISTAR ALBINO RATS

Rosemarinus officinalis L was evaluated for dermal irritation and hair growth stimulation activity in Wistar albino rats. Hair length determination, hair density were recorded for the evaluation.

Dermal irritation study

The gels were non-irritant upon application on to the shaved rat skin (Table No.3). The control and experimental rats showed no signs of tremor, convulsion and reflex abnormalities. The food intake per day had also found normal during 7 days repeated dose dermal toxicity evaluation. There was no reddening, erythyma or lesions observed on skin applied with any of the two gels.

Hair length determination

The experiment is carried out according to the procedure and the results are tabulated in the Table No.4.

In the present study the formulations containing Rosemary 5% show significant hair growth stimulation property with respect to hair length. Rosemary gel when used show better hair growth as against control from 15th day of treatment which was not significant in standard minoxidil treated group. The gel formulation treated animals showed better hair growth as compared to standard minoxidil treated group.

Hair density

The experiment is carried out according to the procedure and the results tabulated in the Table No.5.

Statistical analysis

Values as expressed in mean ± SEM.

***p < 0.0001 for ANOVA for both parameters like hair length determination and hair density determination.

Discussion

Rosemary is important herb commonly finds in various hair care preparations both in traditional and proprietary formulations available in the market. Although Rosemary herb is common components of herbal hair care preparations, there are no reports on scientific evaluation of Rosemary herb on hair growth stimulation. Hence an attempt to formulate and evaluate the effects of Rosemary herb as a gel for hair growth is made herein.

The hydro-alcoholic extract was used for formulation and evaluation of hair growth properties. Besides alcohol is a good solvent for extraction of most of the phyto constituents: hydro-alcoholic extract of rosemary was prepared using 70% alcohol by soxhlation for 8 hrs. The yield of extract was noted.

Phytochemical studies of the drug showed that Rosemary contains phenols, saponins, fixed oils, flavanoids, terpenes. Quantification of phytoconstituents viz: polyphenols, flavonoids and sterols contents in the hydro-alcoholic extract was done by reported colorimetric methods. Rosemary extract showed high flavanoid content (26%w/w) and a polyphenol content (16.5%w/w). Rosemary showed low quantity of sterols. The polyphenol content of rosemary as determined here is higher than earlier reports[35,36]. Flavonoid and sterol content however are not reported for the Rosemary. We have found rosemary extract was rich in flavonoids and presence of polysterols.

As the extract was rich in polar constituents and it was decided to formulate as hydrogel preparation. The prepared formulation was tested for skin irritation and then evaluated for effects on hair growth. The gel was non-irritant upon application on to the shaved rat skin. The control and experimental rats showed no signs of tremor, convulsion, reflex abnormalities and dermal toxicity evaluation. There was no reddening, erythyma or lesions observed on skin applied with gel.

The effects of the formulated gel on hair growth during a 30 day treatment period was observed in Wistar albino rats. Duration for hair growth resumption hair growth on 15^{th}, 20^{th}, 25^{th} and 30^{th} day of treatment and hair density (number of strands/follicles/unit area) on 30^{th} day were the parameters considered. Both treated as well as control rats showed hair growth on 7^{th} day after shaving. The formulation significantly improved hair growth in terms of length and density on 15^{th}, 20^{th}, 25^{th} and 30^{th}day as compared to control. The rate of hair growth(hair length and density) was faster in Rosemary treated group as compared to 2% minoxidil treated group during the 30^{th} day study period. The Rosemary gel shows synergistic effects with respectively rate of hair growth (determined with respect to hair length/time).

Rosemary is reported to improve micro-circulation[r]. Micro-circulation improvement may have an important role in stimulating hair follicles for initiation of hair growth thereby improving hair density.

It is therefore observed that Rosemary is a better hair growth stimulator as it improves both hair density as well as hair length compared to control. The hair growth promoting properties of the Rosemary may be due to their polyphenols, flavonoids and sterol contents. Further studies are required to confirm the role of each of these phytoconstituents in hair growth stimulation.

Table No.1: Qualitative chemical tests of the hydro alcoholic extract of *Rosemarinus officinalis* L

S.No	Chemical constituents	Tests	Rosemary
1	Alkaloids	Meyers test	-ve
		Dragendroff's test	-ve
		Wagner's test	-ve
		Hager's test	-ve
2	Carbohydrates	Molisch's test	-ve
		Benedict's test	-ve
		Fehling's test	-ve
		Barfoed's test	-ve
3	Glycosides	Modified Borntragers test	-ve
		Legal's test	-ve
		Liberman buchard's test	+ve
4	Saponin	Foam test	+ve
5	Fixed oils and fats	Stain test	+ve
6	Resins	Acetone-water test	-ve
7	Phenols	Ferric chloride test	+ve
8	Flavonoids	Alkaline reagents	+ve
		Lead acetate	+ve
		Shinado test	+ve
9	Protein and amino acid	Xanthoproteic test	-ve
		Ninhydrin test	-ve
		Biuret test	-ve
10	Diterpenes and triterpenoids	Copper acetate	+ve
		Tshugajen test	-ve
11	Triterpenes and phytosterols	Salkowski's test	+ve
		Liberman buchard's test	+ve

Note: + ve Indicates presence of phytoconstituents; whereas – ve Indicates absence of phytoconstituents.

Table No.2: Content of total phenols, total flavonoid and total sterol content in hydro alcoholic extract of *Rosemarinus Officinalis* L

S.No	Extracts	Total flavonoids % as quercetin	Total phenols % as gallic acid	Total sterols %
1	*Rosmarinus Officinalis* L	26	16.5	0.96

Table No.3: Data showing the skin irritation of the three gels

S.No	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control	A	A	A	A	A	A	A
2	Rosemary gel (5%)	A	A	A	A	A	A	A

A- No reaction

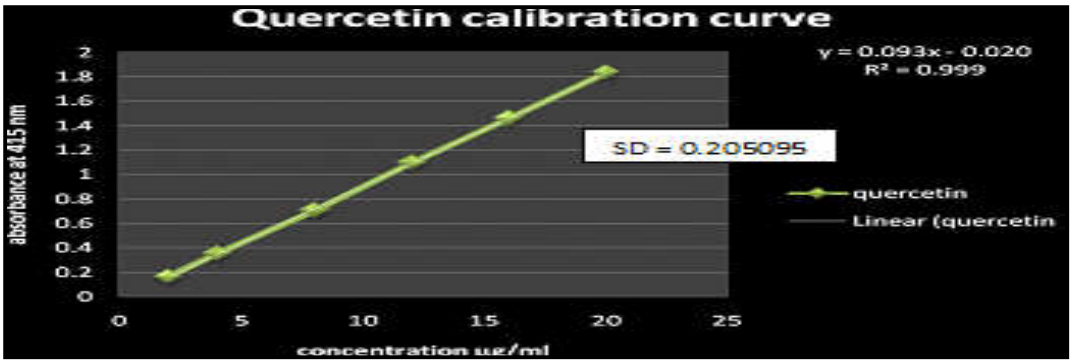
Table No.4: Effects of 2%minoxidil, rosemary on hair growth in wistar albino rats

S.No	Treatment Group	Dose (%)	15th Day	20th Day	25th Day	30th Day
1	Group-1Control	-	5.86±0.93	8.5±0.56	11.7±0.56	14.26±0.360
2	Group-2 Rosemary (5%) gel	5%	7.06±0.8***	9.65±0.88***	14.36±0.44***	16.9±0.239***
3	Group-3 2% minoxidil (1ml)	2%	6.5±0.4*	9.70±0.92***	13.77±0.63***	16.16±0.399***

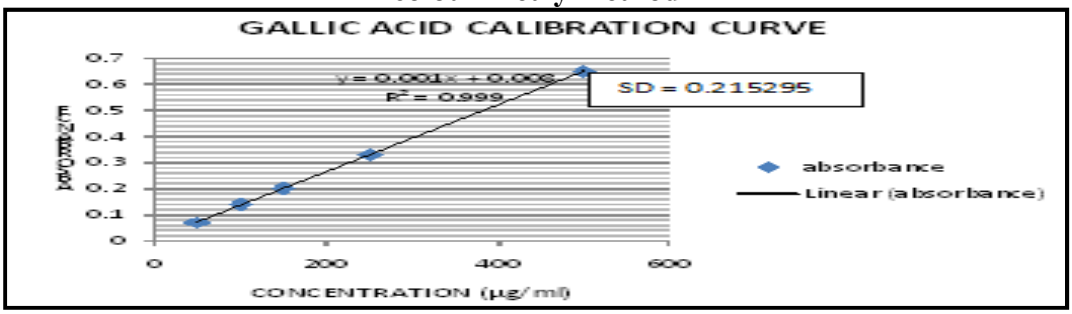
Values mean± SEM, *p < 0.01, **p < 0.05, ***p < 0.0001. Compared to control group by ANOVA (n=25 hairs)

Table No.5: Effects of Control, 2% minoxidil and *Rosemarinus officinalis* LON hair density in wistar albino rats

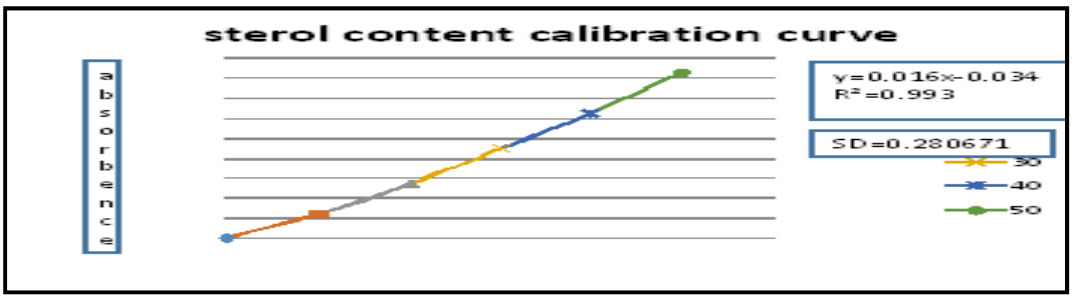
S.No	Treated Group	Dose	Hair density
1	Group-1Control	-	1245±46
2	Group-2 Rosemary (5%) gel	5%	2350***±30
3	Group-3 2% minoxidil (1ml)	2%	1944***±27



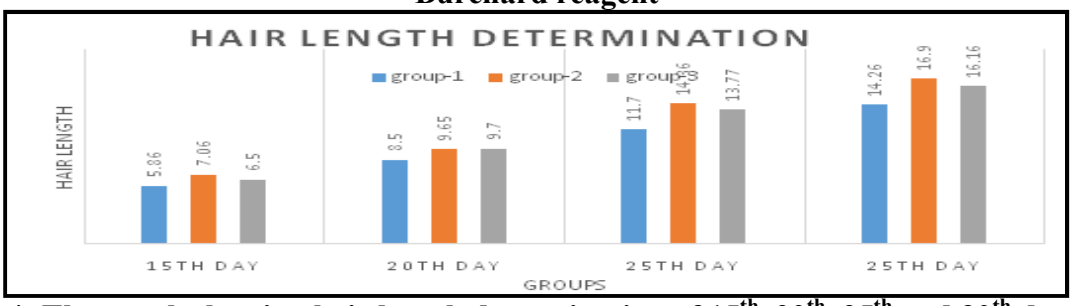
Graph No.1: The calibration curve with standard Quercetin for the determination of flavonoids by $AlCl_3$ colourimetry method



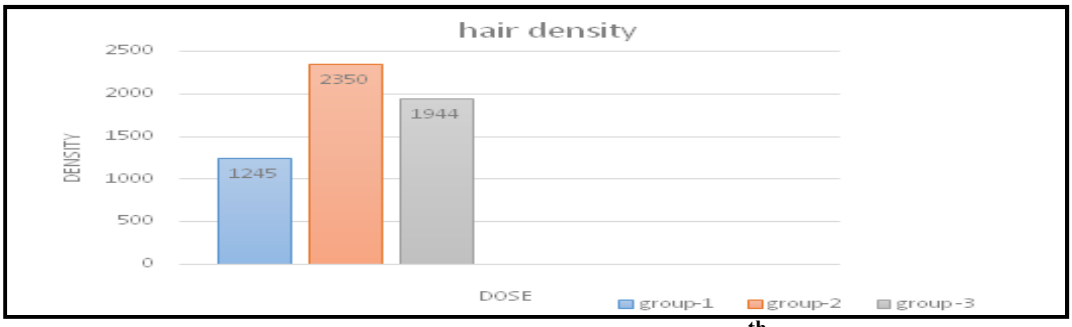
Graph No.2: The calibration curve of gallic acid for determination of polyphenol by Folin Ciocalteau method



Graph No.3: The calibration curve of Cholesterol for determination of sterol content using Liberman-Burchard reagent



Graph No.4: The graph showing hair length determination of 15th, 20th, 25th and 30th day treatment



Graph No.5: The graph showing hair density after 30th day of the treatment

Values mean± SEM, *p < 0.01, **p < 0.05, ***p < 0.0001. Compared to control group to formulation treated groups by ANOVA (n=6).

CONCLUSION

The present study aimed at formulation of *Rosemarinus officinalis* L and evaluation of the same for hair growth. Extraction of *Rosemarinus officinalis* was done by soxhlation and yielded 7.12%. The hydro-alcoholic extracts of Rosemary showed presence of phenols, fixed oils, flavonoids. Quantification of phyto-constiuents showed that Rosemary contained 16.5% polyphenol, 26% flavanoids and 0.96% sterol contents respectively. Gel formulations were tested for short term skin irritation; all the gels were free from skin irritation. Study for effects of the formulated gel on hair growth showed that Rosemary promoted hair growth both in terms of hair length as well as hair density in a 30 day study. This is the report on scientific evaluation of *Rosemarinus officinalis* L for hair growth promoting activity. Thus our study reveals *Rosemarinus officinalis* L to be good hair growth stimulators, their hydro alcoholic extract may be formulated as hydro gels with satisfactory physico-chemical parameters, however more studies on stabilizing the formulation for phytochemical composition needs to be done.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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