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Investigation of hair growth promoting ability of herbal gel containing *Zingiber officinale*

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ABSTRACT

In East Asia, *Zingiber officinale* has been used traditionally to stimulate hair growth. In the present investigation, extraction of the powdered rhizomes of *Zingiber officinale* was carried out using alcohol: water (1:1), water, and alcohol. Preliminary pharmacognostic evaluation of various phytoconstituents was done by various chemical tests. Obtained extracts were studied for their antifungal activity against *Aspergillus niger* and *Candida albicans.* The further herbal gel of 5% of extracts of *Zingiber officinale* and 5% minoxidil (standard) were prepared using carbopol 934 as a base. Prepared formulation (F1-F4) were evaluated for various parameters like pH, viscosity, spreadability, homogeneity, stability studies, drug content, *in vitro* release, skin irritation, and *in vivo* hair growth activity. Amongst the prepared formulations, a gel containing hydroalcoholic extract (F1) showed better spreadability, homogeneity, optimum viscosity, nearly neutral pH, faster release, produced negligible erythema and edema, was stable at different temperature. It was found that gel (F1) showed better results on the length of the hair strand (28mm), which was nearly equivalent to the standard solution of minoxidil (25mm). Conclusively, the hydroalcoholic extract of *Zingiber officinale* could be used to improve hair growth.

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INTRODUCTION

Alopecia is a very common dermatological problem seen around the world and can occur at any age, which results in psychological distress. Herbs are invaluable gifts from nature, and recently the world market is flooded with herbal cosmetics. Various reasons reported for alopecia include metabolism, hormones, heredity, and side effects of antineoplastic and immunosuppressant drugs (Regupathi, 2017). Indian Traditional medicine system acclaims a number of herbal formulations for hair growth promotion, but lack of sound scientific support and knowledge limits their usage.

Ginger is commonly known as monocotyledonous perennial plant *Zingiber officinale* belonging to family Zingiberaceae (Mascolo *et al.*, 1989). *Zingiber officinale* having Gingerol, zingiberene, and the shogaols is well known for its nutraceutical value. Anti-oxidant, carminative, stimulant, antiemetic, and antibacterial property of rhizome of *Zingiber officinale* is reported (Evans, 2009; Kikuzaki and Nakatani, 1993).

Pungency to the rhizome of *Zingiber officinale* is

imparted by 6-GN, while other GN includes 4-, 8-, 10- and 12-GN (Evans, 2009). Potassium, magnesium, and vitamins present in the roots of rhizome are responsible for making stronger, healthier hair, which can ultimately reduce hair fall. Moreover, it is rich in fatty acids such as linoleic acid, which nourishes the hair and helps in preventing the thinning of hair. The texture of the hair gets smoothen by providing essential nutrients. (Sanwal *et al.*, 2010; Zadeh and Kor, 2014).

Extract of ginger possesses a natural antiseptic property, which helps in the prevention of dandruff by killing the microbes and also helps to inhibit the growth of yeasts, flacks, and itchiness caused by dandruff. Circulatory agents present in rhizome improve blood flow to the scalp and ultimately improves hair growth. The bactericidal and anti-inflammatory properties of ginger are responsible for treating different scalp issues caused by harmful microbes (Reddy *et al.*, 2014). In consideration of earlier studies, it was found that the *Zingiber officinale* is responsible for inhibiting the 5α-reductase activity and thereby promote hair growth (Kumar *et al.*, 2012; Ekwenye and Elegalam, 2005; Patel *et al.*, 2015).

Considering all reported hair growth promotion ability of rhizome of *Zingiber officinale*, in the present investigation, aqueous, alcoholic, and hydroalcoholic were formulated into a gel. The formulated herbal gel was evaluated for antifungal activity and hair growth promotion ability in albino rats.

MATERIALS AND METHODS

Extraction procedure of ginger

Rhizomes of *Zingiber officinale* (30 g) were collected from the local market and dried at room temperature. Aqueous, alcoholic, and hydroalcoholic (1:1) extraction was done in the Soxhlet apparatus. Extracts were filtered, and the filtrate was concentrated under vacuum in a rotavapor (7D, Superfit, India) at 40°C. The hydroalcoholic residue was further dried in a hot air oven at 60°C for 2h. The extracts were immediately tested for phytochemical screening, antifungal activities, and refrigerated until used. (Regupathi, 2017).

Phytochemical screening

Qualitative estimation of alkaloids, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, glycosides, and steroids of hydroalcoholic extract of *Zingiber officinale* was performed (Arunkumar and Muthuselvam, 2009).

Antifungal Activity

Agar well diffusion method was used to test antifungal activity against *Aspergillus niger and Candida albicans.* Microbial inoculum (0.5% v/v) was spread over solidified agar. Borer was used to making wells (10 mm) on solidified agar. The sterile syringe was used to add extracts into the wells. Plates were kept in the refrigerator to facilitate diffusion, afterward, plates were incubated at 37^{o}C for 24 h. The zone of inhibition was read and compared to the zone of inhibition of control well. (Arunkumar and Muthuselvam, 2009; Ogbonna *et al.*, 2014).

Formulation of gels

The greater popularity of hydroalcoholic gel is due to is its effective dispersion ability. Gels containing various extracts were formulated, as shown in Table 1. Carbopol 934 was gently dispersed into a specified quantity of water with continuous stirring to avoid lump formation. Then the hydroalcoholic extract was added to the carbopol mixture with constant stirring. To the homogenous dispersion of herbal extract, glycerine was added, and a neutral pH of gel formulation was obtained with the addition of triethanolamine Regupathi (2017). The formulated gel was evaluated for pH (Ubaid *et al.*, 2016), viscosity (Taksande *et al.*, 2013), spreadability, and homogeneity.

Spredability = $\frac{Weight\ tide\ to\ upper\ slide \times Length\ of\ glass\ slide}{Time\ taken\ to\ separate\ the\ slide\ completely\ from\ each\ other}$

Spreadability

In the house, the apparatus was used to determined spreadability. Apparatus was made up of a wooden block having a pulley at one end. Slip and drag characteristics of the gels were used to measure spreadability. Gel (about 2 g) was placed on the ground slide and fixed on this block. Another glass slide has a hook, and the same dimension of the ground fixed slide was kept over the gel.

Weight of 100 g was placed on a top slide to expel air and to produce the uniform film. The excess gel was scraped off from the edges. Around 20 g weight was applied with the help of string attached to hook, and the time required to cover a distance of 7.5 cm was observed. Short time interval indicates better spreadability, which was calculated by the formula (Dwivedi and Gupta, 2012; Jadhav *et al.*, 2009).

E1-Hydroalcoholic extract. E2-Alcoholic extract. E3-Aqueous extract.

F1- Hydroalcoholic extract gel, F2- Alcoholic extract gel

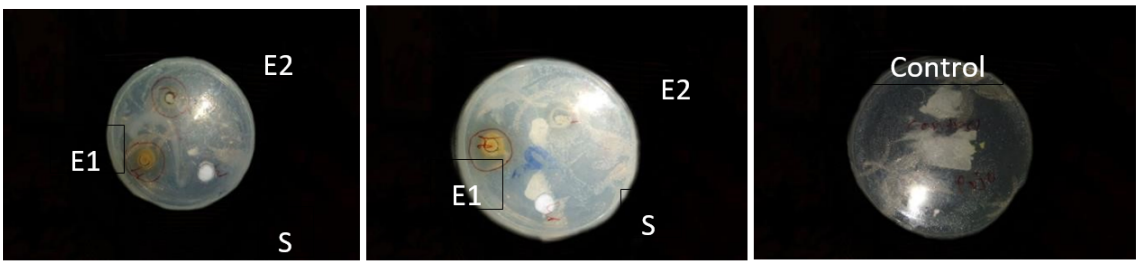
F3- Aqueous extract gel, F4- Standard (Minoxidil gel)

Table 1: Formulation of herbal hair gel

Ingredients	F1 Hydroalcoholic herbal extract(5%)	F2 Aqueous herbal extract(5%)	F3 Alcoholic herbal extract(5%)	F4 Minoxidil(5%)
Carbopol 934 P	2%	2%	2%	2%
PVP	0.5%	0.5%	0.5%	0.5%
Methyl paraben	0.75%	0.75%	0.75%	0.75%
Glycerine	1.3%	1.3%	1.3%	1.3%
PEG	0.25%	0.25%	0.25%	0.25%
Triethanolamine	0.15%	0.15%	0.15%	0.15%

Table 2: Preliminary Phytochemical Screening of Zingiber officinale extract

Plant constituents	Test performed and reagent	Extract		
		E1	E2	E3
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	+	+	-



1.*Aspergillus niger* 2.*Candida Albicans* 3.Control

E1-Hydroalcoholic Extract E2-Aqueous Extract S-Standard (Minoxidil)

Figure 1: Zone of inhibition shown by the above extracts

Table 3: Result of Antifungal Activity

Microorganism used	Zone of Inhibition (mm)		
	Hydroalcoholic extract	Aqueous extract	Minoxidil (standard)
Aspergillus niger	15 ± 0.15	12 ± 0.32	16 ± 0.12
Candida albicans	17 ± 0.13	13 ± 0.17	15 ± 0.18

Table 4: Physicochemical Evaluation

Formulation	Homogeneity	Grittiness	Colour
Hydroalcoholic extract gel	+++	–	Light brown
Aqueous extract gel	+++	–	Light brown
Alcoholic extract gel	+++	–	Light brown
Minoxidil gel	+++	–	White

Table 5: pH, Viscosity, and Spreadability

Formulation	pH(mean ± SD, n = 3)	Viscosity (cp)	Spreadability g.cm/sec
Hydroalcoholic extract gel	6.7 ± 0.01	4731	44.41
Aqueous extract gel	6.6 ± 0.01	4688	41.61
Alcoholic extract gel	6.9± 0.02	4892	42.16
Minoxidil gel	6.6± 0.01	4647	39.94

Table 6: Percent of drug content

Formulations	Drug content (%)
Hydroalcoholic extract gel	96.32
Aqueous extract gel	81.32
Alcoholic extract gel	89.53
Minoxidil gel	92.62

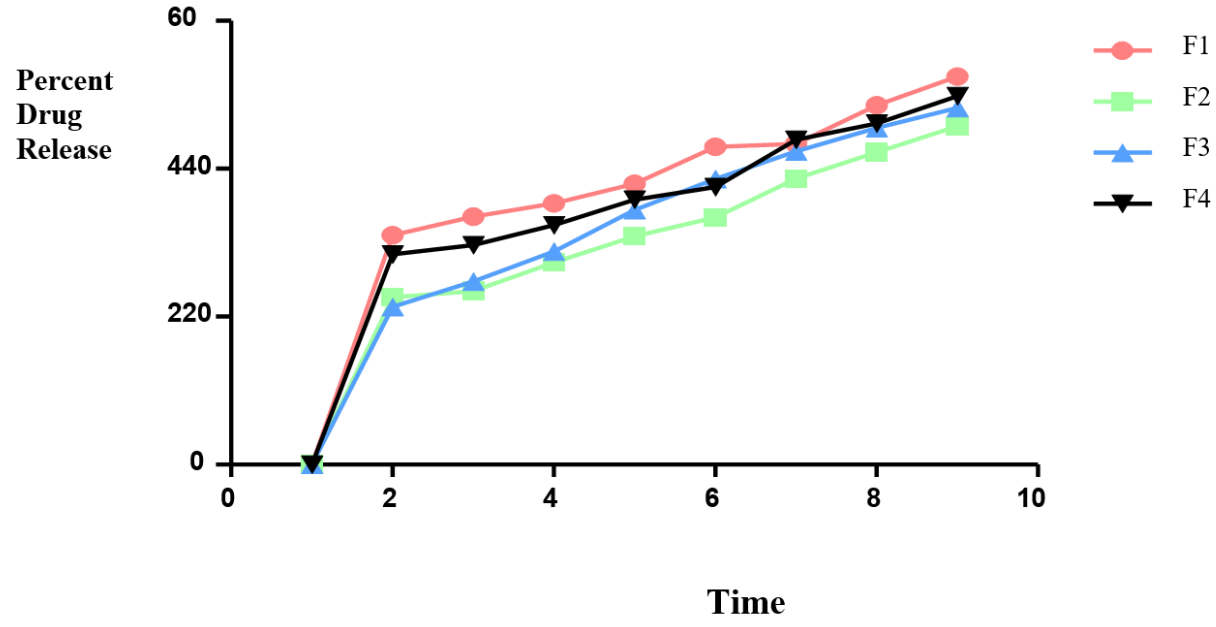


Figure 2: Plot of In-Vitro Drug release

Figure 3: Hair growth of animal after 30 days of Treatment (a, b, c, d: shaved dorsal portion of rats on 0days and A, B, C, D: hair growth promotion on 30^{th} day from hydroalcoholic, aqueous, alcoholic herbal gel extract and minoxidil gel group respectively)

Table 7: Results of Skin Irritation Test

Formulation	Visual observation	
	Erythema	Edema
Hydroalcoholic gel	Nil	Nil
Alcoholic gel	Nil	Nil
Aqueous gel	Nil	Nil
Minoxidil gel	Nil	Nil

Table 8: Effect of Zingiber officinale gel formulation on hair length

Treatment	Mean length of hair in mm		
	10^{th}day	20^{th} days	30^{th} day
Hydroalcoholic extract gel	9 ± 0.2	20 ± 0.2	28 ± 0.2
Aqueous extract Gel	5 ± 0.2	15 ± 0.2	22 ± 0.2
Alcoholic extract gel	6 ± 0.2	14 ± 0.2	20 ± 0.2
Minoxidil gel	7 ± 0.2	17 ± 0.2	25 ± 0.2

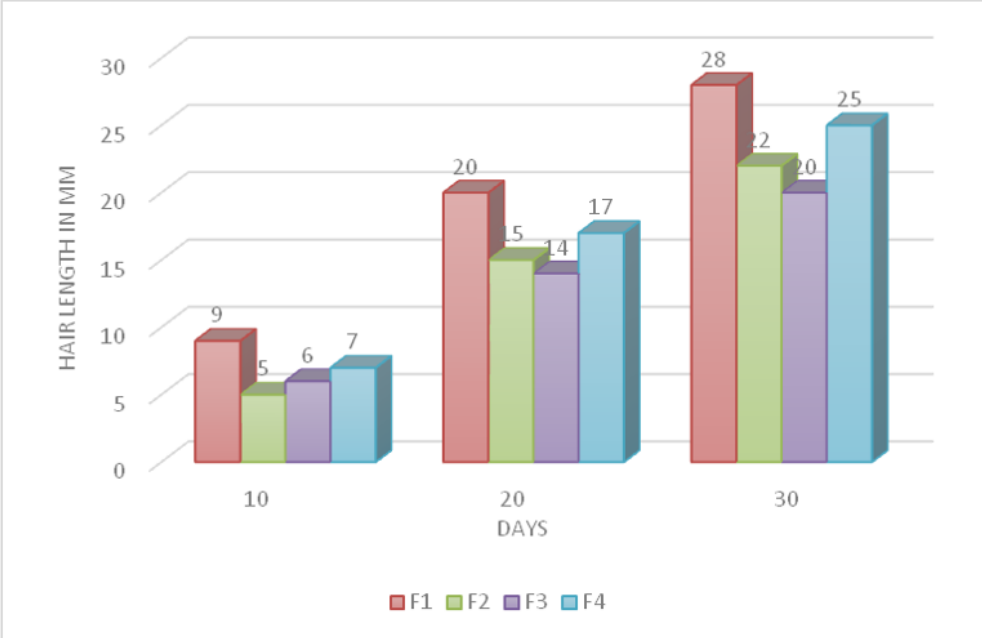


Figure 4: Hair length of Zingiber officinale gel and Minoxidil gel within 30days (F1-Hydroalcoholicgel. F2- Aqueous extract gel. F3-Alcoholic gel. F4- Standard (Minoxidil))

Homogeneity

Visual inspection was done to check homogeneity once gel was set in the container. Appearance, color, clarity, and presence of any aggregates were checked (George and Mathews, 2014; Taksande *et al.*, 2013).

Extract and Drug content

Formulations (1 g) were dissolved in 50 mL of phosphate buffer 7.4. Solutions were kept on a shaker for 2h, subsequently filtered and diluted appropriately. Absorbance was measured by using UV visible spectrophotometer (1700, Shimadzu, Japan) at 281 nm and 288 nm for extract and standard respectively (George and Mathews, 2014; Haneefa *et al.*, 2010; Aly *et al.*, 2013).

In vitro drug release studies

Modified Franz diffusion cell (Murthy lab, Hyderabad, India) was used to study in vitro drug release through a dialysis membrane (cellulose acetate membrane, Sigma Aldrich). The gel equivalent of 10 mg of herbal extract was kept on the presoaked

Table 9: Stability study

Temp	Gel	Viscosity	Appearance
4°	Hydroalcoholic extract gel	+++	+++
	Aqueous extract gel	+++	+++
	Alcoholic extract gel	+++	+++
	Minoxidil gel	+++	+++
25°	Hydroalcoholic extract gel	+++	+++
	Aqueous extract gel	+++	+++
	Alcoholic extract gel	+++	+++
	Minoxidil gel	+++	+++
37°	Hydroalcoholic extract gel	+++	+++
	Aqueous extract gel	+++	+++
	Alcoholic extract gel	+++	+++
	Minoxidil gel	+++	+++

dialysis membrane. Phosphate buffer pH 6.8 was filled in the receptor compartment. The donor compartment was kept in contact with a receptor compartment with the clamps, and the temperature was maintained at 32±0.5 ºC. Stirring in the receptor compartment was done with a magnetic stirrer, and sampling was done at predetermined intervals. After sampling equal volume of phosphate buffer maintained at the same temperature was added in the receptor compartment. The withdrawn sample was diluted appropriately, and absorbance was measured by using a UV visible spectrophotometer (1700, Shimadzu, Japan) at 281 nm and 288 nm for extract and standard, respectively (Parhi and Teja, 2014; Shinde *et al.*, 2012).

Skin irritation test

Rats were divided into four groups and guidelines of the Organization for Economic Co-operation and Development (OECD) test guidelines 404: acute dermal irritation was followed. The dorsal part of the animals was shaved with an electric shaver. Electric shaver was used carefully to avoid skin injury, which would affect the results of skin irritation study. After 3 days of shaving, herbal extracts (hydroalcoholic. aqueous and alcoholic) were applied to respective groups consecutively for three days. After 3 days, the skin was examined visually for erythema and edema (Misal *et al.*, 2012).

In-Vivo Hair growth activity test

Male and female Wistar albino rats (weighing approximately 200-250g) were used for hair growth studies. Rats were housed in standard polypropylene cages along with standard environmental conditions (23°C Temp, 60%RH), fed with a standard diet, and allowed free access to drinking water. The animal requirement was approved by the institutional animal ethics committee (IAEC), and all experiments were conducted as per the norms of the committee for the purpose of the supervision of Experiments on Animals, India. The quantitative model developed for the study of hair growth was used. Rats were divided into 4 groups having 5 animals in each group. The approximate 2cm^2 area of the dorsal portion of all the rats was shaved. Hydroalcoholic, aqueous and alcoholic extract herbal gel of *Zingiber officinale* was applied to the shaved dorsal portion of rats of group 1, 2, and 3, respectively, and standard minoxidil gel was applied to group 4 once a day for 30 days. During the treatment of 30 days, hair growth pattern was observed and recorded (Purewal *et al.*, 2008; Banerjee *et al.*, 2009; Another *et al.*, 2014; Datta *et al.*, 2009) (Regupathi, 2017).

Stability studies

Gel formulations were stored at 4°, 25°, and 37° C for 3 months to check stability, and the determined quantity of gel was removed periodically (0.5, 1, 2, and 3 months). Withdrawn samples were evaluated for drug content, physical appearance, and viscosity (Das *et al.*, 2011; Kaur *et al.*, 2010).

RESULTS AND DISCUSSION

Phytochemical Screening

Results of Preliminary Phytochemical Screening of *Zingiber officinale* extracts are shown in Table 2.

Antifungal Activity

Zone of inhibitions obtained by various extracts and their measurements are shown in Figure 1 and Table 3 Respectively. Antifungal activity of *Zingiber officinale* extracts indicated that it was effective against *A. niger and C. Albicans* with *the* prominent

zone of inhibition. All the formulations were found to be homogeneous, free of grittiness without phase separation with light brown viscous, creamy preparation. The pH value for the herbal hair growth-promoting gel formulations was recorded using digital pH meter are shown in Table 5 and was found to be in the range of 6.5 $\pm$ 0.01 to 6.9 $\pm$ 0.02. It was found that all the formulations were near-neutral pH. Viscosities of gel ranged from 4688 to 4892 cps that were in the usual range of topical gel and were easily spreadable. The spreadability of the formulations was found to be in the range of 39.94 to 44.41.

Physicochemical evaluation

Results of homogeneity, grittiness, and color determinations of various formulations are shown in Table 4. All the formulations were found to be homogeneous, free of grittiness without phase separation with light brown viscous, creamy preparation. The pH value for the herbal hair growth-promoting gel formulations was recorded using digital pH meter are shown in $ and was found to be in the range of 6.5 $\pm$ 0.01 to 6.9 $\pm$ 0.02. It was found that all the formulations were near-neutral pH. Viscosities of gel ranged from 4688 to 4892 cps that were in the usual range of topical gel and were easily spreadable. The spreadability of the formulations was found to be in the range of 39.94 to 44.41.

Extract and Drug content

The percent extract contents and minoxidil contents obtained in the formulated gels are depicted in Table 6.

The drug content of the gel preparations was found to be uniform among various batches prepared within the range from 81.32 to 96.32%. The drug content determination conjointly showed that the drug was uniformly distributed throughout the gel.

***In vitro* drug release studies**

In vitro drug release from the different gel, formulations are shown in Figure 2 . The drug release from all the formulations at the end of 8 h was almost the same and ranged between 44.77$\pm$0.52 and 52.47$\pm$0.95%. It can be observed that Hydroalcoholic gel released at a faster rate followed by an alcoholic, aqueous, and standard Minoxidil gels

Skin Irritation Test

The skin irritation test of herbal extract gels (F1-F4) was compared against Minoxidil gel, a standard irritant, and results are shown in Table 7. Negligible erythema and edema have shown by herbal gel extract formulations indicate an absence of any dermatological reactions. We can also conclude that formulated herbal gel extracts were well tolerated by the rats.

***In-vivo* Hair Growth promotion study**

Hair length determination

Longer hair strands (28 mm) at the end of the 30^{th} day were observed with hydroalcoholic herbal extract gel compared to aqueous (22 mm), alcoholic herbal extract gel (20 mm), and minoxidil gel (28 mm). Greater effect with hydroalcoholic herbal gel extract on hair growth might be due to the conversion of hair follicles from telogen to anagen phase of the hair growth cycle. Other probable reasons are explained by (Uno and Kurata, 1993) that the proliferation of epithelial cells of the hair follicle and dilation of scalp blood vessels might improve hair growth. (Paus, 2006) reported the conversion of short vellus hair to long terminal hair growth due to the anagen phase prolongation. Results of hair growth promotion are shown in Table 8, Figure 3, Figure 4.

Stability Studies

Stability study of all formulations was performed according to International Conference on Harmonization (ICH) guidelines and are reported in Table 9 No separation and precipitation observed with the developed formulations indicate the stability of herbal extract gel at different temperatures. Stability behavior was also observed with pH, viscosity, appearance, and homogeneity.

CONCLUSION

In this study, herbal gels were formulated using extracts of ginger and evaluated for their potential as an effective topical formulation for the hair growth-promoting activity. The results showed that the hydroalcoholic gel of *Zingiber officinale* extract exhibited good pH, acceptable viscosity, and was stable at all the temperature. Moreover, the animal study assured that the prepared hydroalcoholic gel has a promising effect on the promotion of hair growth without any side effects. Thus, it can be revealed that the herbal plant could be a better alternative for future formulations.

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