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Formulation And Evaluation Of Herbal Hydro Gel For Hair Growth

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Abstract
Hydrogels are cross linked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids. In the current research hydrogel formulations of *Hibiscus Rosa Sinensis* were developed by incorporating the gelling agent. Various evaluation parameters like swelling index, pH, viscosity, increased with the increase in the concentration of the polymer. Spreadability decreased with the increase in the polymer concentration. Skin irritation studies revealed that there was no irritation or redness on the skin on the application of the formulations on the skin. Stability study suggested that the formulation was stable at refrigerated condition as compared to room temperature. Anti microbial studies suggested that the formulations were more effective in hair disorders than the coconut oil. All the combined results revealed that the formulation second was the best formulation, which was subjected to TEM analysis, particle size and Polydispersity index determination and anti oxidant study. Morphology study by TEM revealed that most of the particles were spherical. Antioxidant study of the formulation is supposed to attribute to the hair growth.

Keywords: Hydrogels, hydrophilic, *Hibiscus Rosa Sinensis*, TEM

1. INTRODUCTION

Hair has been a sign of beauty and a contribution to an individual's personality since time immemorial. Alopecia (baldness), a dermatological disorder, is a common problem of cosmetology as well as primary health practice. It is common throughout the world and has been estimated to affect between 0.2% and 2% of the world's population.[1-3] The clinical severity of alopecia in a patient may not be a good indicator of a subsequent downturn in quality of life or psychological well-being of the patient. Drugs which claim to treat hair loss target a steadily growing, multi-billion dollar market worldwide. Great expectations are associated with pharmaceutical hair loss management, but still there is no radical improvement in the availability of more precise therapies.[4]

Hydro gels are hydrophilic polymeric network of three dimensional cross linked structures that absorb substantial amount of water.[5] Cross linking facilitates insolubility in water because of ionic interaction and hydrogen bonding.[6] It also provides required mechanical strength and physical integrity to the Hydro gels.[7] Various combinations of polymers are made into hydro gels formulations to investigate their potential as a drug delivery system.

The main goal of the study was to provide a formulation to the people which would be more effective than synthetic drugs, cheaper than available formulations, should be easily available, having minimal side effects, showing faster hair growth and having better penetration into scalp.
In the present research, the hydrogel formulations were prepared of the flowering part of Hibiscus Rosa Sinensis, followed by the evaluation parameters. The ultimate aim was to provide a formulation with better hair growth properties.

2. MATERIAL AND METHODS

2.1 MATERIALS

Hibiscus Rosa Sinensis flowers were obtained from Herbal powders and herbal oil, 36, Kaulagarh road, Dehradun and Carbopol-934, Triethanolamine, propylene glycol were obtained from Grey Scientific, Ambala.

2.2 PREPARATION OF HYDROGEL OF HIBISCUS ROSA SINENSIS

2.2.1 PREPARATION OF EXTRACT

The extract was prepared by infusion technique in which Fresh infusions were prepared by macerating the drug for a short period of time with mixture of boiling water and ethanol and after 24 hours the filtration was done which gave us the hydro alcoholic extract and this extract was further used for the preparation of hydrogel.[8] The composition of the hydro gel is tabulated in the following table (table no. 1)

Table No. 1: Composition of hydro gel

Formulation Code	Carbopol 934	Propylene Glycol -400	Triethanolamine	Distilled water	Extract
F - 1	1 gram	Quantity sufficient	1.2 ml	Up to 100 ml	100 mg
F - 2	1.5 gram	Quantity sufficient	1.2 ml	Up to 100 ml	100 mg
F - 3	2 gram	Quantity sufficient	1.2 ml	Up to 100 ml	100 mg

2.2.2 PREPARATION OF HYDROGEL

Carbopol-934 was dispersed in 50 ml of distilled water with continuous stirring. Propylene glycol 400 was added gradually to form a homogenous mass. Further hydro alcoholic extract of Hibiscus Rosa sinensis (100 mg) was mixed to the above mixture and volume was made up to 100 ml by adding remaining part of distilled water. All the ingredients were mixed properly with carbopol 934 to form a smooth hydro gel. Finally triethanolamine was added drop wise to the formulation for the adjustment of required pH of about 4.5- 5.5, to form a hydro gel of required consistency. The prepared hydro gel was subjected to various evaluation parameters.

2.3 EVALUATION OF HYDROGEL

2.3.1 PHYSICAL EVALUATION

The prepared hydrogel formulations were inspected for visual (colour, homogeneity, consistency), olfactory (smell), tactile (texture, feel upon application such as grittiness, greasiness, stickiness, smoothness, stiffness and tackiness) characteristics.[9]

2.3.2 pH

pH of the hydro gel was determined with the help of the digital pH meter which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 min prior to taking the reading at room temperature. The test was done in triplicate.[10]

2.3.3 VISCOSITY

Viscosity of gel was measured by using Brookfield viscometer with spindle no. S 64. The test was performed in triplicate and average was taken.[11]

2.3.4 SPREADABILITY

The formulated hydrogel was placed over the glass plate of 20cm × 5cm. Another glass plate of the same dimension was placed on the top of the gel such that the formulation was sandwiched between the two slides by placing a weight of 100g uniformly on the slides. The weight was removed and the excess of gel was scrapped off. Two slides in position were fixed to a stand at a 45° angle without the slightest disturbance so that only the lower slide was held firmly by the clamp, allowing the upper slide to slip off freely with the help of 20g weight tied to the upper slide. The time taken for the upper slide to separate away from the lower glass plate under the direction of the weight was noted as Experiment was done in triplicate and spreadability was calculated as follows:

$S = \frac{M}{T} \times L$

Where, S = Spread ability, L =Length of the glass plate. M=Weight tied to the upper plate, T = Time taken (sec).[12]

2.3.5 SKIN IRRITATION STUDY

This evaluation was performed on healthy human volunteers including both male and female candidates. About 0.5 gram of herbal hydrogel was applied to an area of approximately 6cm^2 of skin. At the end of the exposure period of 1 hour, skin was checked for any irritation or redness.[13]

2.3.6 SWELLING STUDIES

To determine the swelling index of prepared topical gel, 1 gm of gel was taken on petri dish and then placed separately in a 50 ml beaker containing 10 ml distilled water. Then the samples were removed from beakers at different time intervals and put it on dry place for
some time after it re weighed. Swelling index was calculated as follows Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100

Where, (SW) % = Equilibrium percent swelling, Wt = Weight of swollen gel after time t, Wo = Original weight of gel at zero time.[21]

2.3.7 DETERMINATION OF PARTICLE SIZE AND POLY DISPERSITY INDEX BY THE USE OF ZETA SIZER

The hydro gel formulations were characterized for their size and Polydispersity index were also analysed by Zeta Sizer [Beckman Coulter] after the appropriate dilution with the suitable diluent.

2.3.8 TRANSMISSION ELECTRON MICROSCOPY

The morphology of the hydrogel was analysed by the help of transmission electron microscope [FEI Technai G^2 F20 Netherlands]. The sample was suspended in 1 ml of distilled water and taken on the carbon coated copper grid and finally negative stained with 2 % Phosphotungstic acid. Then it was air dried for half an hour and analysed at 120 kV.

2.3.9 ANTIOXIDANT STUDIES

2.3.9.1 TOTAL ANTIOXIDANT ASSAY BY PHOSPHOMOLYBDENUM METHOD

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based

on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity was calculated by the method described by Prieto *et al*. (1999). 0.3 mL of sample (second formulation) was combined with 1 mL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ^{0} C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution was measured at 695 nm against blank in UV spectrophotometer. The results are expressed as no. of ascorbic acid equivalents.[15,20]

2.3.9.2 HYDROGEN PEROXIDE SCAVENGING ASSAY

The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Sample (20–60 µg/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of Hydrogen peroxide scavenging was calculated as follows: % scavenged $H_2O_2 = {}^{Ai-At} \times 100$

At Where Ai is the absorbance of control and At is the absorbance of test.[14–15]

2.3.10 STABILITY STUDIES

The drug loaded hydrogels were subjected to stability studies for a period of 3 months at room temperature [30 ± 2 ^{0} C, refrigerated condition i.e. [4 ± 2 ^{0} C] and at accelerated condition [40 ± 2^{0} C, 75 % R.H.] After 1 month, 2 months and 3 months of storage, the formulations was subjected to test for physical stability and pH.[16–17]

2.3.11 ANTI- MICROBIAL STUDIES

Material and method

Test organisms and Inoculums:

Gram positive: Staphylococcus aureus

Gram negative: E coli

Standard: Coconut oil

Media: -Dehydrated nutrient agar media was used and was prepared in distilled water.

The composition of the media was as given below.

1) Agar 15.0%
2) Peptic digests of animal tissue 5.0%
3) Sodium chloride 5.0%
4) Beef extract 1.5%
5) Yeast extract 1.5%
6) pH 7.4 ± 0.2 at 25^{o}C
7) Distilled water 1000 ml

The medium was autoclaved at 15 lbs per square inch pressure at 121^{o}C

2.11.1 PREPARATION OF MEDIA

Dehydrated nutrient agar media (28gm) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely.

2.11.2 STERILIZATION OF MEDIA

The conical flask containing the nutrient agar medium was plugged with the help of nonabsorbent cotton bung. The mouth of the conical flask and the cotton bung were properly covered with aluminium foil. The medium was then sterilization by autoclaving at 15 lbs per square inch pressure for 20 minutes.

2.11.3 METHOD: CUP AND PLATE METHOD

The sterile nutrient agar medium at a temperature between 40°to 50°C was immediately poured into the sterile Petri plates to give a depth of 3 to 4 mm, by placing the plates on a level surface. The plates were then allowed to solidify. Each plate was then inoculated with0.1ml of the solution of test organisms prepared in water for injection. The wells in each plate were bored in the centre that was filled with hydro gels. The plates were then incubated at 37° C for 24h. After incubation, zone of inhibition (inhibition around each well) was measured and this value was taken as an indicator for the antimicrobial activity.[18-19]

3. RESULTS AND DISCUSSION

3.1 PHYSICAL EVALUATION

Hydro gel formulations were found to be translucent in nature with ethanolic odour, smooth feel on application and homogenous and the results of the appearance of the hydrogel is shown in table no. 2.

Table No. 2: Results of physical evaluation of hydrogels

S No.	Formulation Code	Appearance	Feel on Application	Odour
1.	F – 1	Pinkish red	Smooth	Ethanolic
2.	F – 2	Pinkish red	Smooth	Ethanolic
3.	F – 3	Pinkish red	Smooth	Ethanolic

3.2 pH

The pH of all the three formulations was in the range of the pH of the scalp i.e. 4.5 to 5.5 which implies that the formulations can be applied to the scalp and the results are expressed as mean ± standard deviation which is tabulated below in table no. 3.

Table No. 3: Results of pH of hydrogels

S No.	Formulation Code	pH
1.	F – 1	5.08 ± 0.0077
2.	F – 2	5.11 ± 0
3.	F – 3	5.17 ± 0.065

3.3 VISCOSITY

Viscosities of the hydrogels were measured by the Brookfield viscometer in centipoise. Formulation third had maximum viscosity followed by second and first and the results of the viscosity of all the three formulations are expressed in table no. 4.

The data represented that with the increase in the concentration of polymer viscosity was increased.

Table No. 4: Results of viscosity of hydrogels

S No.	Formulation Code	Viscosity (cp)
1.	F – 1	14333
2.	F – 2	49000
3.	F – 3	872000

3.4 SPREADABILITY

All the prepared gels using different polymers in different concentrations were spreadable. The formulation first showed the maximum spreadability followed by second and third and the results are tabulated in table no. 5. Spreadability decreased with the increase in the concentration of the polymer.

Table No. 5: Results of spreadability of hydrogels

S No.	Formulation Code	Spreadability (g.cm/sec)
1.	F – 1	94.49 ± 1.04
2.	F – 2	78.99 ± 1.96
3.	F – 3	54.04 ± 0.608

3.5 SKIN IRRITATION STUDY

All the three formulations were subjected to skin irritation study on voluntary individuals. No formulation showed any sign of skin irritation and redness as observed in figure no. 1 which implies that the formulations are not allergic to the skin.

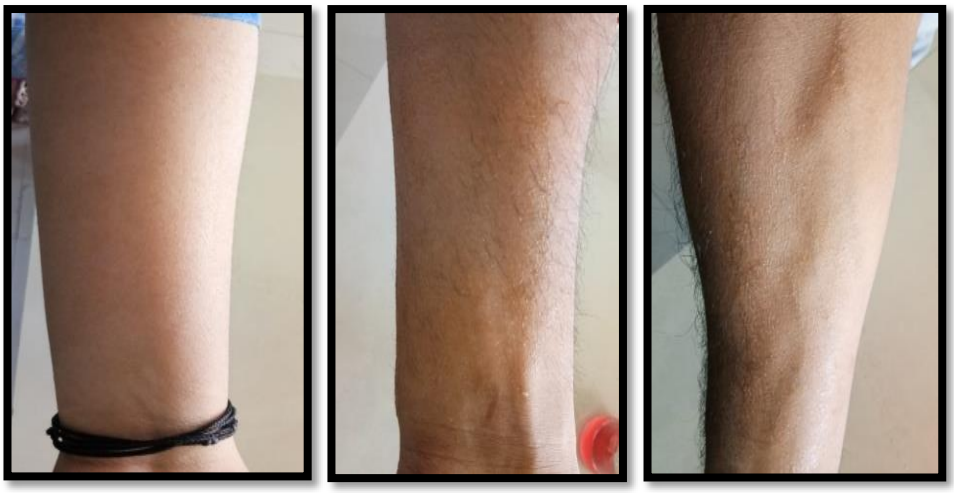


Figure No. 1: Hydrogels subjected to skin irritation study.

3.6 SWELLING STUDIES

Hydrogel can swell thous and of time then its dry weight of the hydrogel. The release of drug from hydrogel particles depends on the swelling behaviour. As the hydrogel swells, the network pores open and drug release occurs. Swelling studies of hydrogels were done as dynamic equilibrium

study. The results are tabulated in table no. 6 and graphically represented in the figure no. 2. Formulation third was having maximum swelling index followed by second and first. So, from this observation from the evaluation parameter, it may be concluded that as the quantity of the polymer increased, the swelling ability of the formulations also increased.

Table No. 6: Results of percentage swelling index of hydrogels

Time (hours)	F – 1	F – 2	F – 3
0	0	0	0
1	13.9 ± 5.45	30 ± 8.16	40 ± 8.16
2	33.3 ± 4.71	43.3 ± 4.64	53.33 ± 4.64
3	36.6 ± 4.71	56.6 ± 4.71	63.33 ± 4.64
4	43.3 ± 4.69	66.6 ± 4.71	76.67 ± 4.64
5	70 ± 8.16	83.3 ± 4.69	86.67 ± 4.71

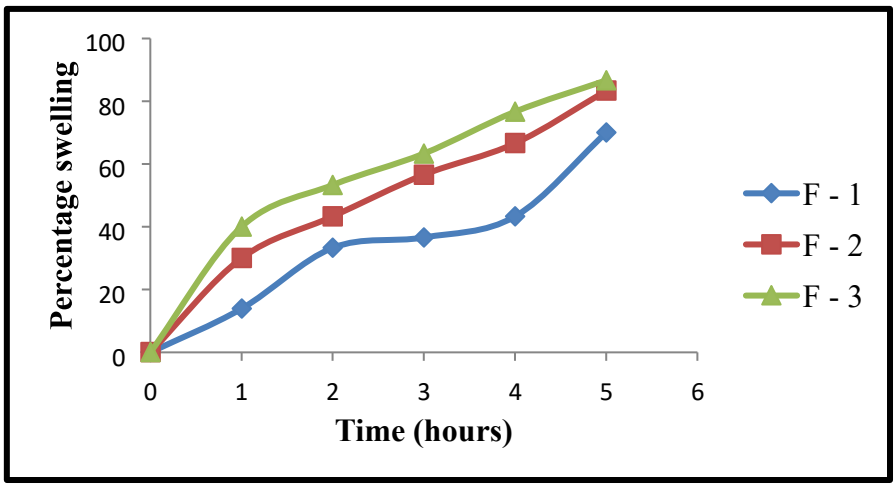


Figure No. 2: Percentage swelling index v/s time profile of formulations F – 1, F – 2 and F – 3.

Based on the above evaluations formulation first was less viscous and third was more viscous and second formulation was having optimum viscosity required for gel. Also second formulation showed good spreadability, optimum for the gel. pH and swelling behaviour was also optimum for the second formulation. So we choose second formulation for the further study.

3.7 DETERMINATION OF PARTICLE SIZE AND POLY DISPERSITY INDEX BY THE USE OF ZETA SIZER

The hydrogel formulation was characterized for their size Polydispersity index by using Zeta Sizer [Beckman Coulter]. Results of average particle size and Polydispersity index were obtained from instrumental based calculation system. Average particle size of hydrogel was found to be 242.1 nm and Polydispersity index (P.I) was found to be -0.464 as shown in Figure: 3.

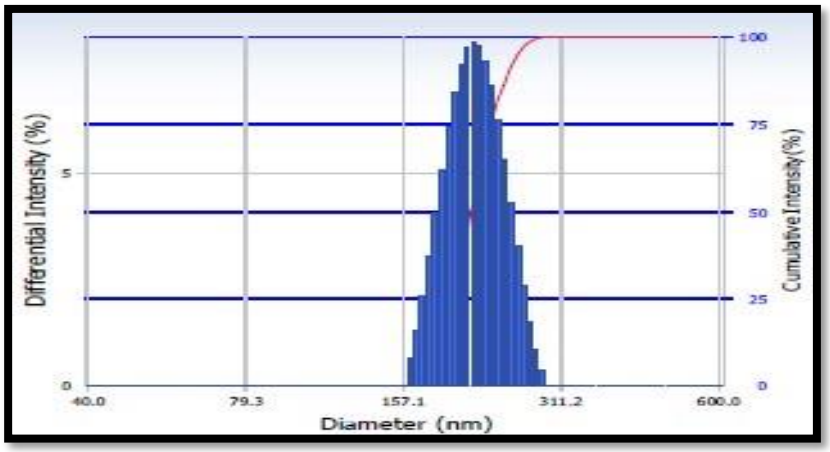


Figure No. 3: Particle size of hydrogel (F – 2) formulation by Zeta Sizer.

3.8 TRANSMISSION ELECTRON MICROSCOPY

The morphology of the hydrogel was analysed by the help of the transmission electron microscope [FEI Technai G^2 F20 Netherlands]. The results are shown in figure no. 4 and 5. Most of the particles were spherical with only few irregular shaped particles.

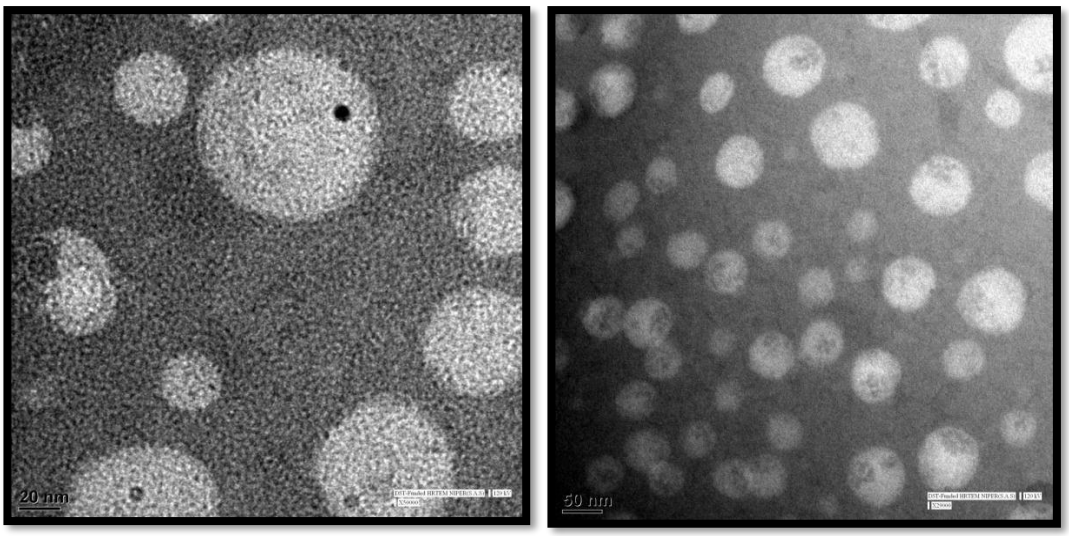


Figure No. 4: TEM images of hydrogel (F – 2)

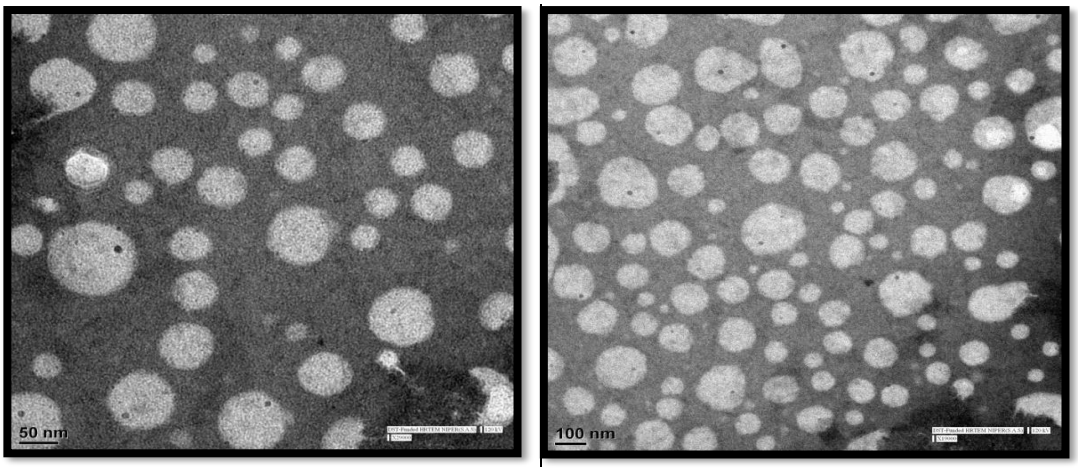


Figure No. 5: TEM images of hydrogel (F – 2)

3.9 ANTIOXIDANT STUDY

3.9.1 TOTAL ANTIOXIDANT ASSAY BY PHOSPHOMOLYBDENUM METHOD

The total antioxidant activity of the hydrogel was evaluated by phosphomolybdenum method. Standard curve was plotted for concentration ranging from 20 to 100 µg/ ml. The results are expressed in table no. 7 and graphically shown in figure no. 7. The antioxidant activity increased with the increase in the concentration of hydrogel which was indicated by the formation of green colour after the incubation. The antioxidant activity is directly proportional to the colour intensity.

The solution having less concentration of hydrogel was less intense and the solution with higher concentration of hydrogel was more intense. The green colour formation took place because of reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH.

Table No. 7: Observations for Total Antioxidant assay by phosphomolybdenum method of F-2 formulation (mean ± standard deviation)

S No.	Concentration (µg/ ml)	Antioxidant assay (µg/ ml Ascorbic acid equivalent)
1.	20	33.62±1.41
2.	40	46.88±1.89
3.	60	64.16±1.75

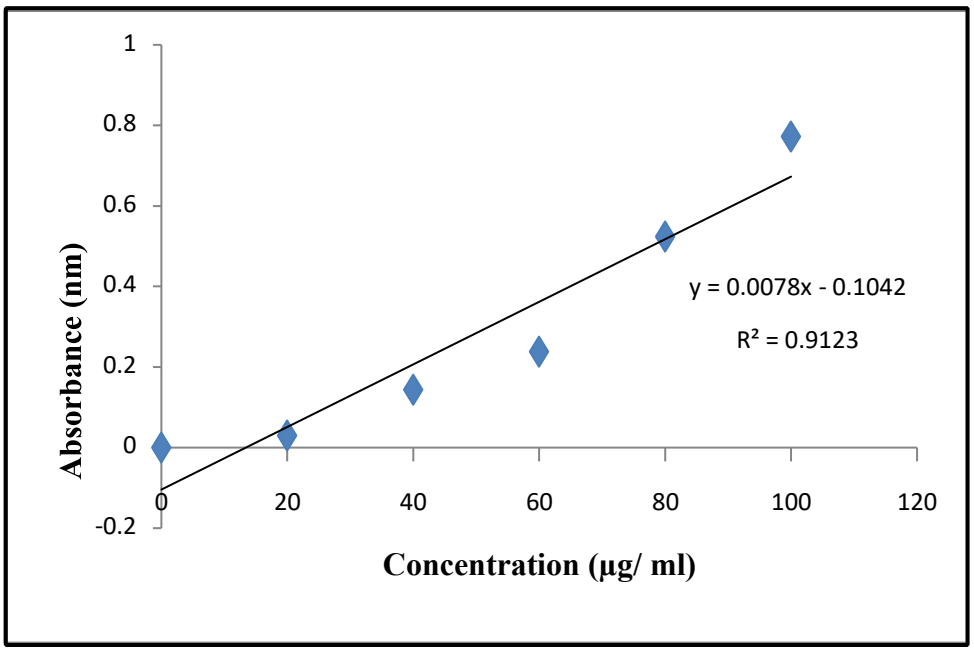


Figure No. 6: Standard plot of ascorbic acid

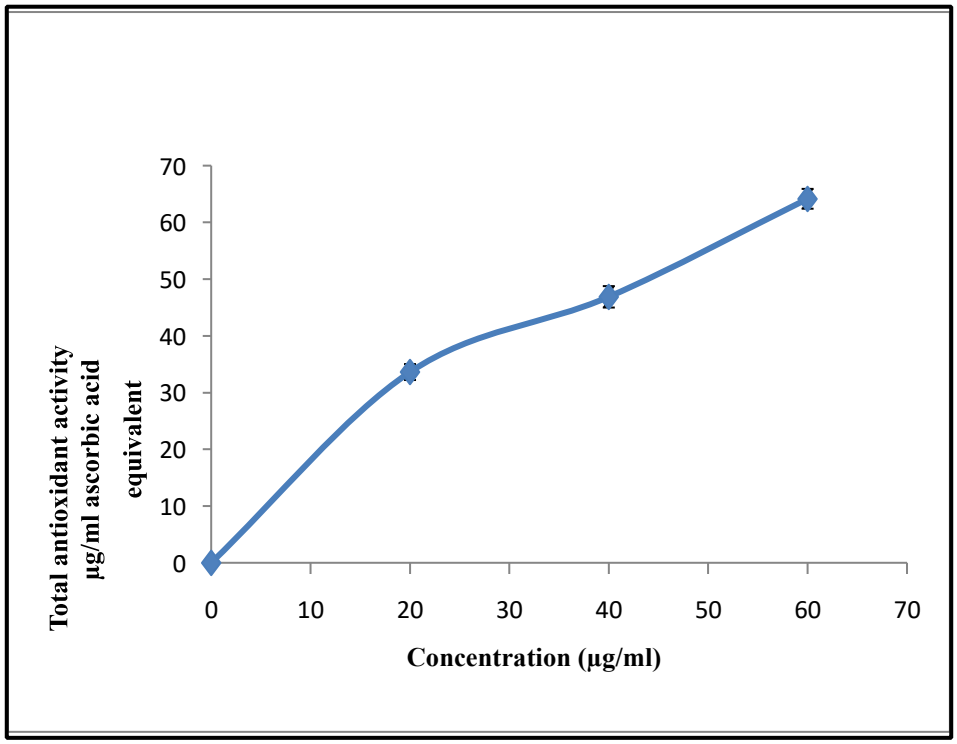


Figure No. 7: Graphical representation of total antioxidant activity by phosphomolybdenum method (F- 2 formulation)

Figure No. 8: Figure showing test tubes before and after incubation

3.9.2 HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The hydrogen peroxide scavenging activity increased with the increase in the concentration of the hydrogel. The results are shown in the table no. 8 and graphically expressed in figure no. 9.

Table No.8: Observations for Hydrogen peroxide scavenging activity of F-2 formulation (mean ± standard deviation)

S No.	Concentration (µg/ ml)	Percentage scavenged hydrogen peroxide
1.	20	24.18±1.17
2.	40	35.88±1.08
3.	60	62.916±0.95

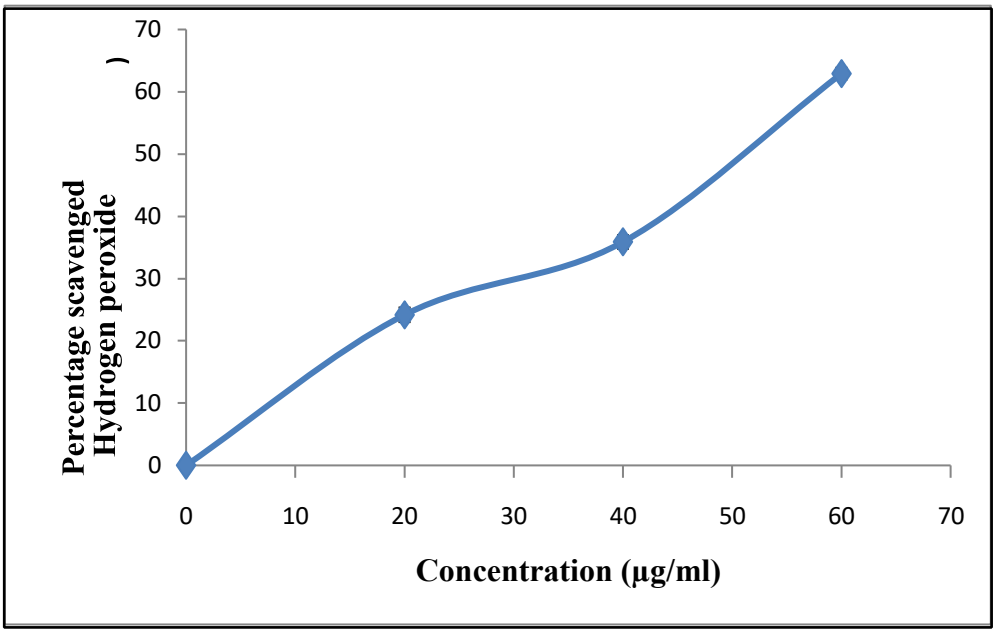


Figure No. 9: Graphical representation of hydrogen peroxide scavenging activity of F-2 formulation

RELATION OF ANTIOXIDANT ACTIVITY AND HAIR GROWTH

Our formulation showed antioxidant activity and according to Ahmad *et al* the antioxidant activity contributes to the hair growth activity. Semalty *et al* also demonstrated that there is direct correlation between antioxidant activity and hair growth. Beoy L. A. *et al* also demonstrated that the antioxidants reduce lipid per oxidation and oxidative stress in the scalp and can be used in the hair growth. So, owing to the antioxidant activity our formulation can be a potent hair growth agent. [22-24]

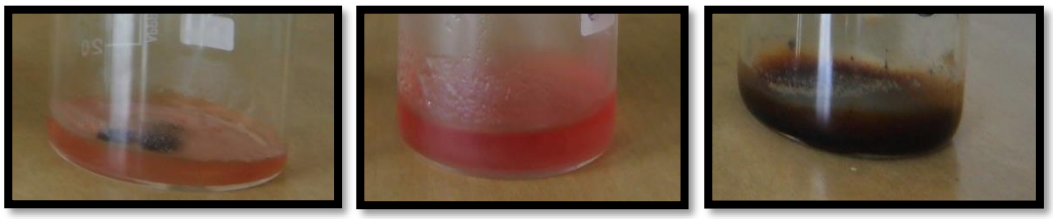
3.14 STABILITY STUDIES

The stability studies are very important evaluation parameter and they were performed in order to study whether the formulation can bear changes in temperature, humidity etc. So, the stability studies were done for 3 months. The formulations were stable up to 2 months and appeared as it is but after 3 months, the formulations kept at the room temperature and at the accelerated condition started to change in appearance (figure no. 10) while those kept in the refrigerator were as it is, which concludes that the formulations were more stable in the refrigerator.

So the freezing temperature would be ideal for the storage of our herbal hydrogels. Also, the pH was increasing with the time. The detailed study and the results are shown in table no. 9 and 10.

Table no. 9: Effect of Temperature on Stability of Hydro gel (physical appearance)

EFFECT OF TEMPERATURE ON STABILITY OF HYDRO GEL			
Temperature	PHYSICAL APPEARANCE (VISUAL)		
	After 1 month	After 2 months	After 3 months
At room temperature	No change	No change	Blackish pigment appeared
At refrigerated condition	No change	No change	No change
At accelerated condition	No change	No change	Blackish pigment appeared



(a) (b) (c)

Figure No. 10: Physical appearance at (a) room temperature, (b) refrigerated temperature and (c) accelerated temperature.

Table no. 10: Effect of Temperature on Stability of Hydro gel (pH)

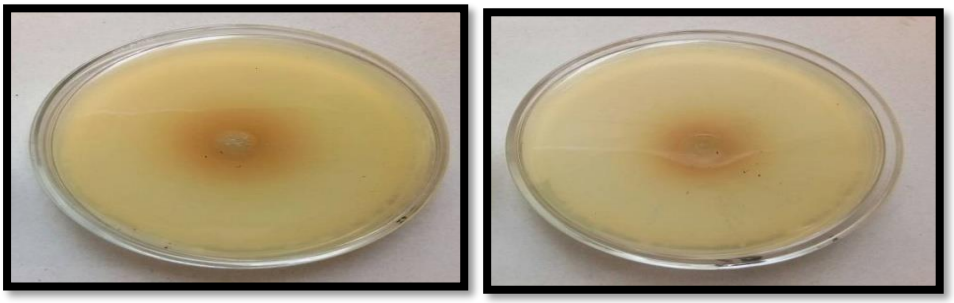
EFFECT OF TEMPERATURE ON STABILITY OF HYDRO GEL			
Temperature	pH		
	After 1 month	After 2 months	After 3 months
At room temperature	5.33	5.44	5.59
At refrigerated condition	5.11	5.18	5.27
At accelerated condition	5.48	5.49	5.70

3.15 ANTI – MICROBIAL STUDY

The antimicrobial activity of hydro gels was found to be better than standard coconut oil as the standard coconut oil did not show any zone of inhibition while the hydrogel showed significant zones of inhibition as shown in figure no. 11, 12, 13, 14 and results are tabulated in table no. 11. So, our hydrogel formulations (all of three) were significantly better than the standard used i.e. coconut oil.

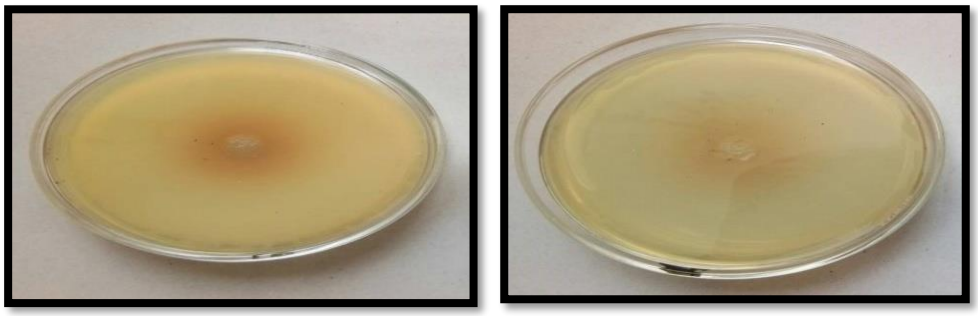
Table No. 11: Anti – microbial activity of formulations and standard coconut oil

S No.	Formulation applied	E Coli	Staphylococcus aureus
1.	F – 1	2.5 cm	2.4 cm
2.	F – 2	2.5 cm	2.4 cm
3.	F – 3	2.3 cm	2 cm
4.	Coconut oil	0 cm	0 cm



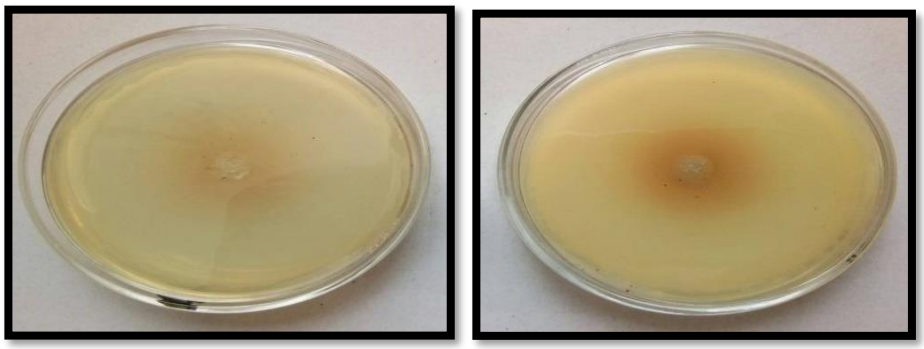
(a) (b)

Figure No. 11: Zone of inhibition against E Coli (a) and staphylococcus aureus (b) of F–1 formulation.



(a) (b)

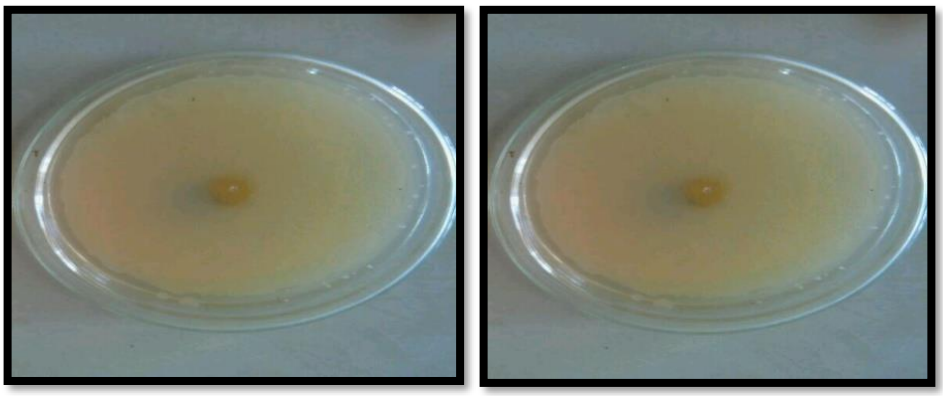
Figure No. 12: Zone of inhibition against E Coli (a) and staphylococcus aureus (b) of F-2 formulation



(a) (b)

Figure No. 13: Zone of inhibition against E Coli (a) and staphylococcus aureus (b) of F-3 formulation.

All the three formulations (F – 1, F – 2 and F – 3) showed the inhibition (as seen by the zone of inhibition in all the plates) of 2 cm or greater as compared to the coconut oil which showed no inhibition which is shown in figure no. 14 below. So, our hydro gels formulations are better than the coconut oil and can be used for the growth of the hair as well as various hair disorders.



(a) (b)

Figure No. 14: Zone of inhibition against E Coli (a) and staphylococcus aureus (b) of coconut oil.

4. CONCLUSION

In the current research hydrogel formulations were developed by incorporating the gelling agent. Various evaluation parameters like swelling index, pH, viscosity, increased with the increase in the concentration of the polymer. Spreadability decreased with the increase in the polymer

concentration. Skin irritation studies revealed that there was no irritation or redness on the skin on the application of the formulations on the skin. Stability study suggested that the formulation was stable at refrigerated condition as compared to room temperature and accelerated conditions. Anti microbial studies suggested that the formulations were more effective than the coconut oil. All the combined results revealed that the formulation second was the best formulation, which was subjected to TEM analysis, particle size and poly dispersity index determination and antioxidant activity. Morphology study by TEM revealed that most of the particles were spherical. Antioxidant activity is supposed to attribute to the hair growth activity.

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