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DEVELOPMENT AND ASSESSMENT OF AN ANTI-DANDRUFF HERBAL GEL FOR ADULT SEBORRHEIC DERMATITIS

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ABSTRACT

Dandruff is a shedding of dead skin cells from the scalp and is suffered by almost 50% of the population and causes significant discomfort. The severity can range from mild scaling to dry skin to severe scaling. Malassezia furfur is considered to be the cause of dandruff. Dandruff may also be caused by changes in humidity, trauma, seasonal changes or emotional stress. The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing *Emblica officinalis.* Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, and *Zingiber officinale.* Eight batches (F1, F2, F3 F4, F5, F6, F7 and F8) of Herbal Antidandruff gel were formulated. The gel formulation was designed by using aqueous, aqueous extracts in varied concentrations along with different polymer. The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. The results showed the stability study were performed for the selected formulation (F8) by both the technique as per the ICH guidelines. The gel was subjected to stability study at 40°C±2°C and 75±5% RH, samples were withdrawn on 1 month, 2 month, 3 month and analysed. The result shown that the product is stable for 3 months without change in physical changes. Since the antimicrobial studies has given encouraging results in enhancing the antidandruff activity of F8 formulation, it is concluded that the F8 Herbal antidandruff gel may be subjected to further *in-vivo* and clinical trials.

KEYWORDS: *Emblica officinalis.* Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, *Zingiber officinale*, Gel, Evaluation.

INTRODUCTION

Dandruff is a very common non-contagious hair problem, nearly affecting person irrespective of age. Medically it is defined as pityriasis simplex capitis– shedding of dead skin from the scalp. It may be–dry or grease. Dry dandruff appears silvery and white while greasy flakes appear pale yellowish and may have an unpleasant smell.[1] Historically there have been multiple other descriptive names reflecting the fungal cause of this condition, such as pityriasis simplex and pityriasis capitis (referring to *Pityrosporum*) and furfuracea (referring to *Malassezia furfur*).[2,3] It is a common embarrassing disorder which effects 5% of the global population.[4,5] Dandruff affects the aesthetic value and causes the itching and keratinocytes play major role in the expressions and the generation of immunological reaction during dandruff formation.[6,7] The severity of dandruff may fluctuate with season as a often worsen in winter. Dandruff is common scalp condition that producing the irritating white flakes and itchy scalp. Excessive drying of skin and over-activity of oil gland known as seborrhea.[8-9] To overcome all these side effects an attempt been made to formulate and evaluate Polyherbal antidandruff gel to minimize all these side effects and to show rapid action on Dandruff.[10] Dandruff is a skin condition that mainly affect the scalp symptoms include flaking and sometimes mild itchiness. it can result in social or self-esteem problem. A more severe form of the condition, which includes inflammation of the skin is known as seborrrhoeic dermatitis.[1] The herbs selected for this work were *Emblica officinalis.* Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, and *Zingiber officinale* are reported to have significant antifungal and anti-inflammatory and antimicrobial activities. The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction, prompted us formulate a polyherbal preparation. The combination is used in order to enhance the Dandruff.[2, 3] Dandruff is a common scalp disorder affecting almost half of the population at the pre pubertal age and of any sex and ethnicity. Pityriasis simplex capillittii (Commonly known as dandruff) is the shedding of dead skin cells from the scalp. The word dandruff is of Anglo Saxon origin, a combination of 'tan' meaning 'tetter' and 'drof'

meaning dirty. Dandruff can be considered aesthetically displeasing and often causes itching. Dandruff is the result of a combination of factors. Some of these factors are well studied, whereas others have not been thoroughly investigated.[4] Flaking is a symptom of seborrhoeic dermatitis. Joseph bark notes that "redness and itching is actually seborrheic dermatitis and it frequently occurs around the folds of nose and the eyebrow areas, not just the scalp". Dry, thick, well defined lesions consisting of large, silvery scales may be traced to the less common psoriasis of the scalp. Dandruff scale is a cluster of corneocytes, which have retained a large degree of cohesion with one another and detach as such from the surface of the stratum corneum. The size and abundance of scales are heterogeneous from one site to another and over time. Parakeratotic cells often make up part of dandruff. Their numbers are related to the severity of the clinical manifestations, which may also be influenced by seborrhea. The most common cause of dandruff is probably the fungus Malassezia furfur (previously known as Pityrosporum ovale) this fungus is a lipid dependent, dimorphic yeast like fungus occurring in human skin as an opportunistic pathogen and is responsible for many cutaneous diseases like dandruff, pityriasis versicolor, seborrheic dermatitis, tinea circinata etc. During dandruff, the levels of Malassezia furfur increase by 1.5 to 2 times its normal level. Dandruff is sometimes caused by frequent exposure to extreme heat and cold. The severity of dandruff may fluctuate with season as it often worsens in winter. Other causative factors include family history, food allergies, excessive perspiration, use of alkaline soaps and stress. Even the season of the year can contribute to the problem, cold, dry winters are notorious for bringing on dandruff. Dan druff can also be aggravated by exposure to dust, UV light/harsh shampoos and hair dyes.[8] A gel (from the Latin Gelu-freezing, cold, ice or gelatusfrozen, immobile)is a solid, jelly like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. By weight, gels are mostly liquid, yet they behave like solids due to three dimensional cross linked network within the liquid. It is the cross links within the fluid that give a gel its structure and contribute to stickiness. In this way gels are a dispersion of molecules or particles within the liquid in which the solid is the discontinuous phase and the liquid is the continuous phase.

MATERIALS AND METHODS

Carbopol 934, Carbopol 940, Triaqueousamine, Glycerine, Polyethylene Glycol, Propyl Paraben, Aloe vera gel, *Emblica officinalis.* Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, and *Zingiber officinale were used.*

Collection of plant material

Emblica officinalis. Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, and *Zingiber officinale.* Roscoe were collected from in and around Tiruchirappalli district, Tamilnadu. Collected herbs were authenticated by the Botanist, Dept. of Botany, Dr. HSG central University Sagar.

Preparation of aqueous extract of selected herbs

Collected and selected parts of herbs such as *Emblica officinalis.* Gaertn, *Citrus limonum.* Risso, *Allium sativum.* Linn, and *Zingiber officinale.* Roscoe were washed with distilled water and grinded individually by simple grinding. Then the extract was filtered, centrifuged and used for further studies.

Preparation of herbal antidandruff gel

Measured quantity of propyl paraben, glycerine and weighed quantity of Polyethylene Glycol were dissolved in about 35 ml of water in beaker. Then it was stirred at 100rpm using mechanical stirrer. Carbopol 940 and 934 were added slowly to the respective beaker containing above liquid while stirring. Triethanolamine (Neutralizing agent) was added slowly with stirring till to attain gel structure. Required proportions of aqueousextracts *Emblica officinalis, Citrus limonum, Allium sativum, Zingiber officinalis* and *Aloe barbadensis* were added to the prepared gel and stirred continuously to form proper gel.

Table 1: Formulation of herbal antidandruff.

S. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
1	*Emblica officinalis*	0.5ml	-	-	0.5ml	0.5ml	-	-	0.5ml
2	*Citrus limonum*	-	0.5ml	-	0.5ml	-	0.5ml	-	0.5ml
3	*Allium sativum*	-	-	0.5ml	0.5ml	-	-	0.5ml	0.5ml
4	*Zingiber officinalis*	-	-	0.5ml	0.5ml	-	-	0.5ml	0.5ml
5	*Aloe barbadensis*	-	-	0.5g	0.5g	-	-	0.5g	0.5g
6	Carbopol 940	0.30g	0.30g	0.30g	0.30g	-	-	-	-
7	Carbopol 934	-	-	-	-	0.30g	0.30g	0.30g	0.30g
8	Polyethylene Glycol	7g	7g	7g	7g	7g	7g	7g	7g
9	Triethanolamine	0.6g	0.6g	0.6g	0.6g	0.6g	0.6	0.6g	0.6g
10	Propyl Paraben	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g
11	Glycerine	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
12	Water q.s	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml

Evaluation methods of formulation

Physicochemical evaluation of herbal antidandruff gels

Gels were evaluated for their clarity, pH, homogeneity, spreadability, viscosity, drug content, extrudability, *in-vitro* diffusion studies, release kinetics, antimicrobial screening, skin irritation test and *ex-vivo* studies by using standard procedure. All studies were carried out in triplicate and average values were reported.

Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid, clear, very clear (glassy).

pH

2.5 gms of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of dispersion was measured by using digital pH meter (Elico).

Homogeneity

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

Spreadability

It was determined by wooden block and glass slide apparatus. For the determination of spreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 minutes. Weight (50 gm) was added to pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plates was taken as measure of Spreadability (S). Spreadability was calculated by using the formula:

$S = ML/T$

where, S = Spreadability, M = Weight tide to upper slide, L = Length moved on the glass slide,

T = Time taken to separate the slide completely from each other

Viscosity measurement

Viscosity of the gels was determined using a Brookfield viscometer, (Brookfield DV-II + Pro viscometer) by using small sample adapter having spindle number SC4-18/13R. The gel was subjected to a torque ranging from 10 to 100 %.

Drug content

The herbal antidandruff gel of 100mg was dissolved in 50 ml of phosphate buffer 7.4. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically.

Extrudability

The extrudability test was carried out by using Pfizer hardness tester. A 15gm of gel was filled in aluminium tube. The plunger was adjusted to hold the tube properly. The pressure of $1kg/cm^2$ was applied for 30 sec. The quantity of gel extruded was weighed. The procedure was repeated at three equidistance places of the tube. Test was carried out intriplicates.

***In-vitro* diffusion study**

Cellophane membrane treatment for permeation study

Cellophane membrane was boiled in the distilled water for 1 hour and washed with fresh distilled water for three times and kept in ethanol for 24 hours. It was treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60^0C followed by acidified with 0.2% sulphuric. Finally the membrane was dipped in boric acid buffer pH till it is used for permeation study.

***In-vitro* diffusion study**

The *in- vitro* permeation rate of selected formulations of gel were evaluated by open ended tube through using pH 7.4 as diffusion medium upto 5 hours studies. The cellophane membrane was tied in one end of the tube and then immersed in the receptor compartment containing 200ml of 7.4 buffer solution which was stirred at 100±10 rpm and maintained at $37^0C \pm 2^0C$. A quantity of 5ml samples were withdrawn from the receptor fluid at the time intervals of 0, 10, 15, 20, 25,30,60,90,120,180,240,300 min. The release of drug was estimated by using spectrophotometer at 273 and 340 nm and 5ml of phosphate buffer of pH 7.4 was replaced immediately each time.

Release kinetics

Data obtained from *in-vitro* diffusion studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=k0t), First order equation{ln (100 – Q) =ln Q – klt}, Higuchi equation (Q=kt1/2), Hixson and crowell model Qt1/3 Vs t and Qt2/3 Vs – Modified root cube equation. Further, to find out the mechanism of drug diffusion, first 60% drug diffusion was fitted in Korsmeyer and Peppas equation (Q=kptn). Where, Q is the percent of the drug diffusion at time t and k0 and kt are the coefficients of the equations and „n" are the diffusion exponent. The „n" value is used to characterize different diffusion mechanism.

The linear equation for zero order drug diffusion plot is:

$Ct = C0 – Kt$

Where, Ct = concentration remaining at time t, Co = original concentration,t = time,

K = diffusion rate

The linear equation for first order diffusion plot is

$$\text{Log } C = \frac{\log C0Kt}{2.303}$$

Hydrophilic matrix tablets contain a water swellable polymer. On [1 – Mt / M] 1/3 =1-kt

Where,
Mt = mass of drug diffusion at time t, M = mass diffusion at the infinite time, K = rate of erosion, t = time. Thus a plot of [1 – Mt / M] 1/3 versus the time will be linear.

Screening of antimicrobial activity of herbal antidandruff gelformulations

Anti-bacterial activity

The Muller Hington Agar Media was prepared, sterilized and used as the growth medium for bacteria culture, 20ml of the sterilized medium was poured into each sterilized petridish, coverd and allowed to solidify. The sterile disc (Whatmann No 2, 6mm diameter) was placed uniformly at equal interval on the inoculated plate. Then it was loaded in the above solidified media. About 200 µ/l of sample was loaded in each disc and incubated at 37^0c for 18-24 hours and the Zone of inhibition was measured by using the ruler

Anti-fungal activity

The Saboraud Dextrose Agar Media was prepared, sterilized and used as the growth medium for fungi culture, 20ml of the sterilized medium was poured into each sterilized petridish, coverd and allowed to solidify. The sterile disc (Whatmann No 2, 6mm diameter) was placed uniformly at equal interval on the inoculated plate. Then it was loaded in the above solidified media. About 200 µ/l of sample was loaded in each disc and incubated at 37^0C for 3-4 days and the Zone of inhibition was measured by using the ruler.

Stability studies

Stability is officially defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process beings at early development phases.

RESULTS AND DISCUSSIONS

Phytochemical studies

The phytochemical studies of *Emblica officinalis, Citrus limonum, allium sativum, Zingiber officinale, Aloe barbadensis* was done. The presence and absence of Phytoconstituents in the aqueous extract of the above sample was shown in **Table.2**.

Table 2: Phytochemical studies.

S. No.	Phytoconstituents	Aqueous extracts				
		Emblica officinalis	***Citrus limonum***	***Allium sativum***	***Zingiberof ficinale***	***Aloe barbadensis***
1.	Alkaloids	+	+	+	+	+
2.	Glycosides	+	+	+	+	-
3.	Saponins	-	-	+	+	+
4.	Tannins	+	+	-	+	+
5.	Phenols	+	+	+	+	-
6.	Reducing sugars	+	+	+	+	+
7.	Amino acids	+	+	+	+	+
8.	Flavonoids	-	+	+	+	+
9.	Terpenoids	-	+	_	+	+
10.	Steroids	+	+	+	-	-

(+) Presence of phytoconstituents (-) Absence of phytoconstituents

Identification of selected herbs

High performance thin layer chromatography (HPTLC)

The HPTLC fingerprinting of Aqueous extract of selected parts of *Emblica officinalis* (AEEO), *Citrus limonum* (AECL) were studied individually. The HPTLC fingerprinting was done. The Peaks viewed at system suitability were shown (**Fig.1 and 10**). The four different chromatograms were obtained and photo documentation was done for individual extracts (**Fig.2 to 5 and 11 to 14**) The 3D display of the chromatogram were obtained and photo documentation were done (**Fig.1 and 18**).

HPTLC finger printing of aqueous extract of *Emblica officinalis* (AEEO)

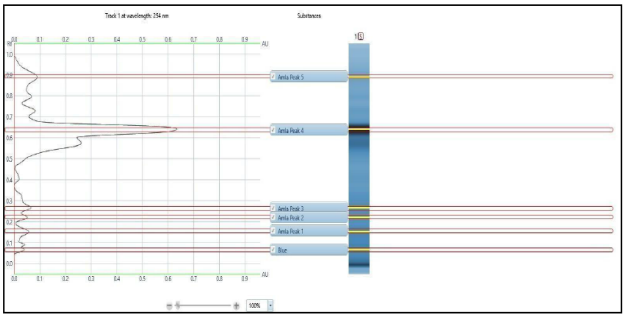

Fig. 1: HPTLC peak at System suitability test for AEEO.

Table 3: Rf values from HPTLC Chromatogram of AEEO.

S. No.	Track No.	Rf value
1.	Tr.1	0.067
2.	Tr.2	0.157
3.	Tr.3	0.222
4.	Tr.4	0.265
5.	Tr.5	0.638
6.	Tr.6	0.893

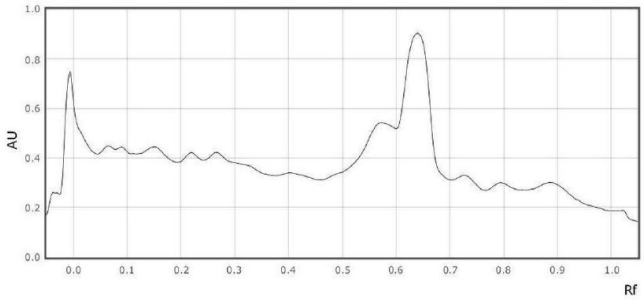

Fig. 2: HPTLC Chromatogram of AEEO at 5 µl Concentration.

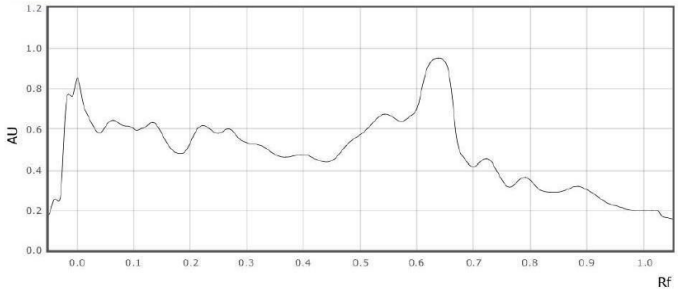

Fig. 3: HPTLC Chromatogram of AEEO at 10 µl Concentration.

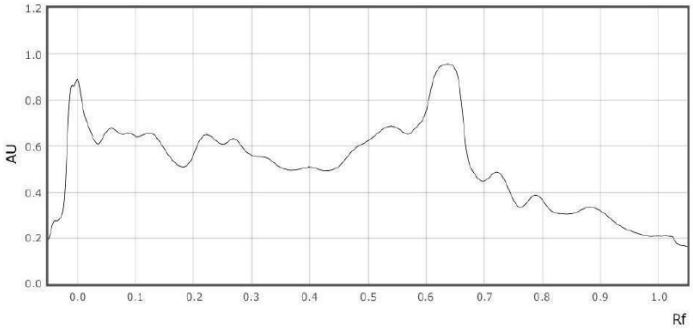

Fig. 4: HPTLC Chromatogram of AEEO at 15 µl Concentration.

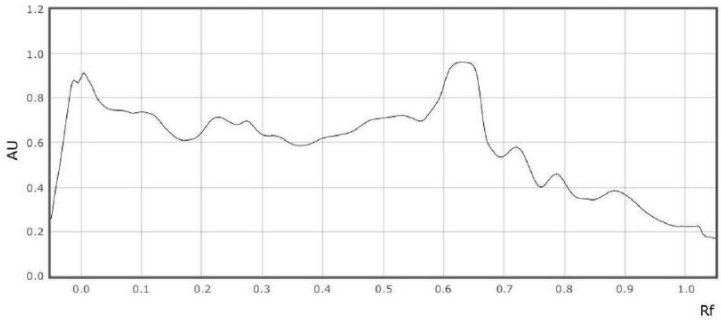

Fig. 5: HPTLC Chromatogram of AEEO at 20 µl Concentration.

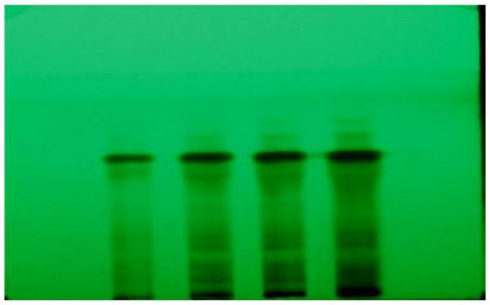

Fig. 6: HPTLC of Amla Extract viewed at 254nm.

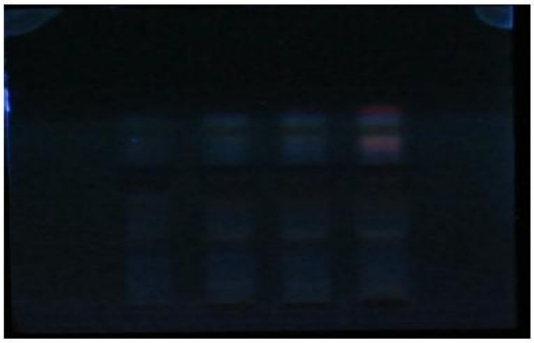

Fig. 7: HPTLC of Amla Extract viewed at 366nm.

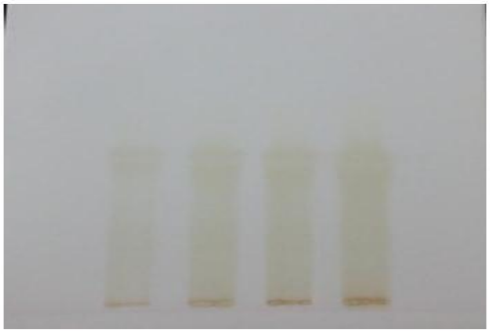

Fig. 8: HPTLC of Amla Extract viewed at Visible Light.

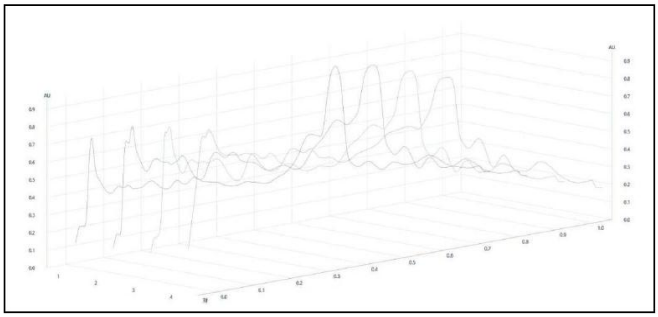

Fig. 9: HPTLC Chromatogram of AEEO (3D) HPTLC.

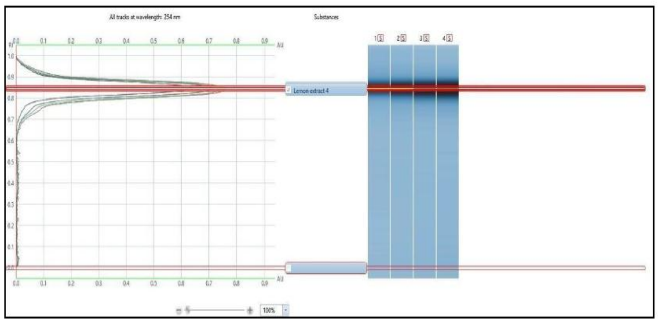

Fig. 10: HPTLC peak at System suitability test for AECL.

Table 4: Rf values from HPTLC Chromatogram of AECL.

S. No.	Track No.	Rf value
1.	Tr.1	0.844
2.	Tr.2	0.846
3.	Tr.3	0.849
4.	Tr.4	0.839

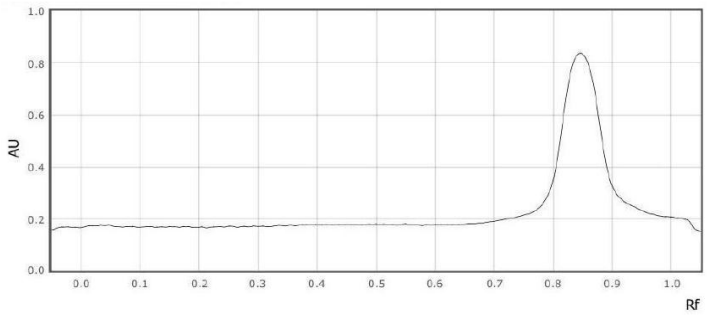

Fig. 11: HPTLC Chromatogram of AECL at 5 µl Concentration.

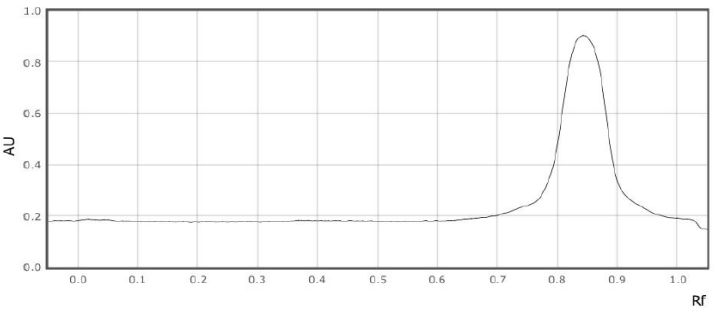

Fig. 12: HPTLC Chromatogram of AECL at 10 µl Concentration.

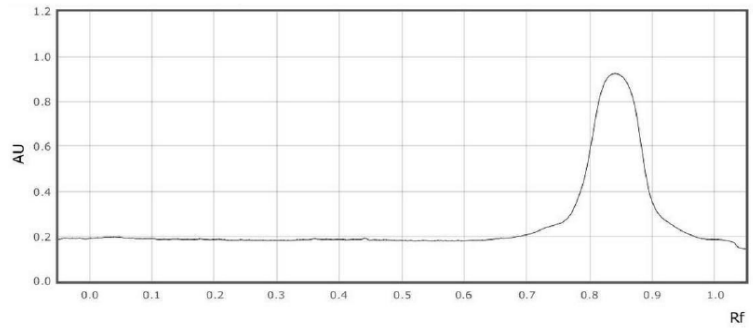

Fig. 13: HPTLC Chromatogram of AECL at 15 µl Concentration.

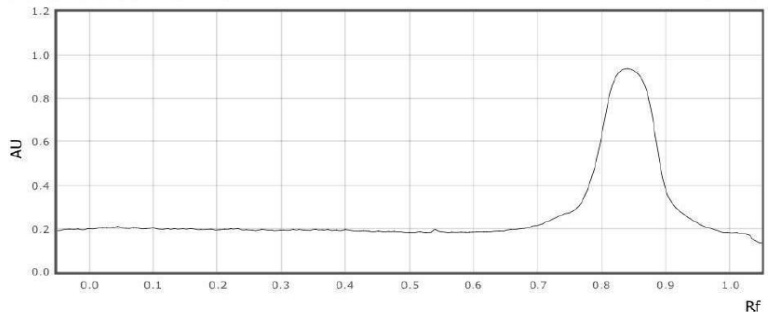

Fig. 14: HPTLC Chromatogram of AECL at 20 µl Concentration.

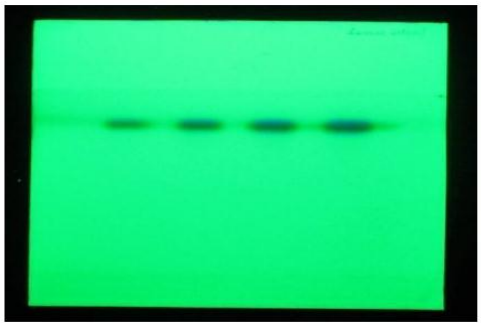

Fig. 15: HPTLC plate viewed at 254nm.

Fig. 16: HPTLC plate viewed at 366nm.

Fig. 17: HPTLC plate viewed at visible light.

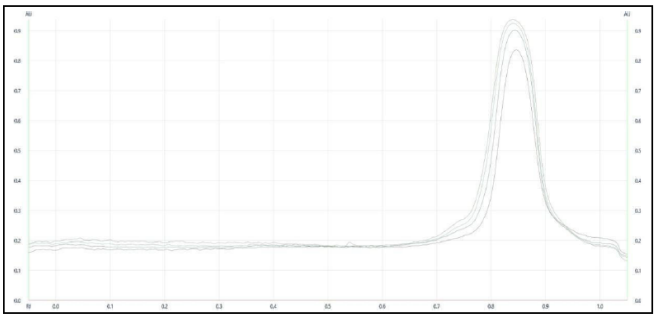

Fig. 18: HPTLC Chromatogram of AECL (3D).

UV Analysis

Absorption maxima (λ max) of Aqueous extract of*Emblica officinalis*. Gaertn

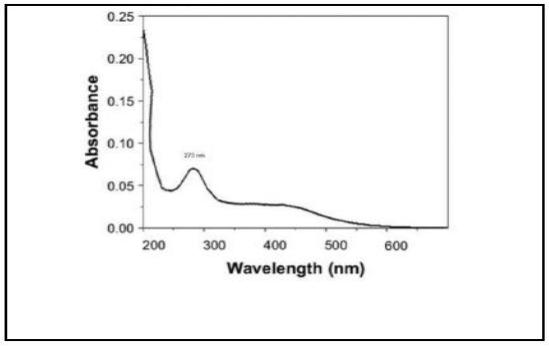

Fig. 19: Absorption maxima (λ max) of Aqueous extract of *Emblica officinalis* Gaertn.

The sharp peak observed at 273nm, further measurements were taken at 273nm.

Standard curve of Aqueous extract of *Emblica officinalis* Gaertn

Table 5: Standard curve of Aqueous extract of *Emblica officinalis* Gaertn.

Concentration(µg/ml)	Absorbance at 273nmAverage ± SD
0	0.000 ±0.001
5	0.257±0.001
10	0.430 ±0.001
15	0.593 ±0.001
20	0.748 ±0.001
25	0.908±0.001
30	1.031 ±0.004

Mean± SD: n= 3

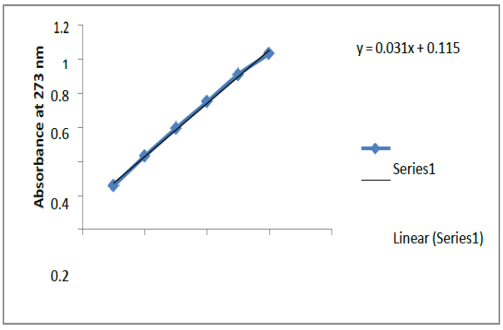

Fig. 20: Standard curve of Aqueous extract of *Emblica officinalis*. Gaertn.

The standard curve has good regression coefficient r^2= 0.997 and it shows thelinearity

Absorption maxima (λ max) of Aqueous extract of *Citrus limonum*. Risso

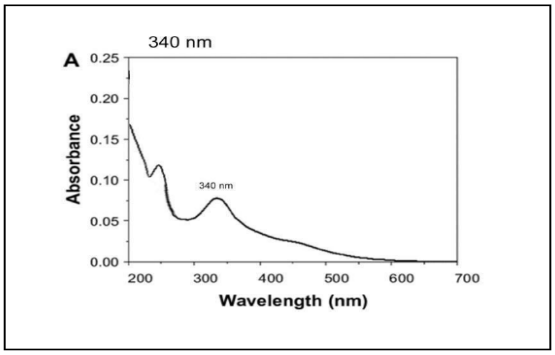

Fig. 21: Absorption maxima (λ max) of Aqueous extract of *Citrus limonum*. Risso.

The sharp peak observed at 340nm, further measurements were taken at 340nm.

Standard curve of Aqueous extract of *Citruslimonum*. Risso

Table 6: Standard curve of Aqueous extract of *Citruslimonum*. Risso.

Concentration (µg/ml)	Absorbance at 340nm Average ± SD
0	0.000 ±0.001
	0.310 ±0.001
10	0.420 ±0.001
15	0.509 ±0.001
20	0.793 ±0.001
25	0.943 ±0.001
30	1.257 ±0.005

Mean± SD: n= 3

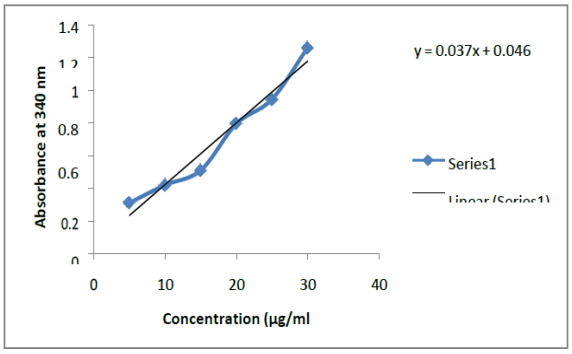

Fig. 22: Standard curve of Aqueous extract of *Citruslimonum*. Risso.

The standard curve has good regression coefficient r^2= 0.961 and it shows thelinearity.

Compatibility study FTIR studies

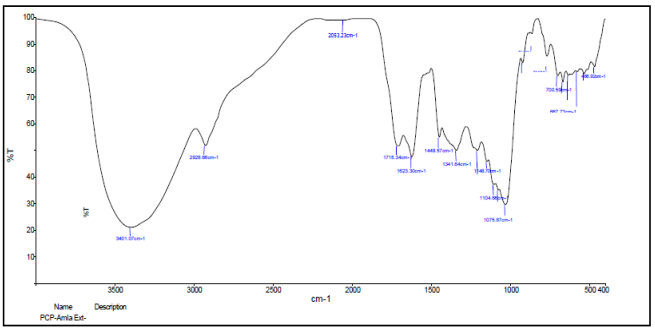

Fig. 23: FTIR Spectrum of Aqueous Extract of *Emblica officinalis.* Gaertn.

Table 7: FTIR Interpretation of Aqueous Extract of *Emblica officinalis.* Gaertn.

Wave number (cm^{-1})	Functional Group
3401.07	O-H stretching
2928.66	C-H Stretching
2053.23	N=C=S Stretching
1715.34	C=O Stretching
1623.30	C=C Stretching
1449.57	C-H Bending
1208.61	C-O Stretching
1075.87	C-O Stretching

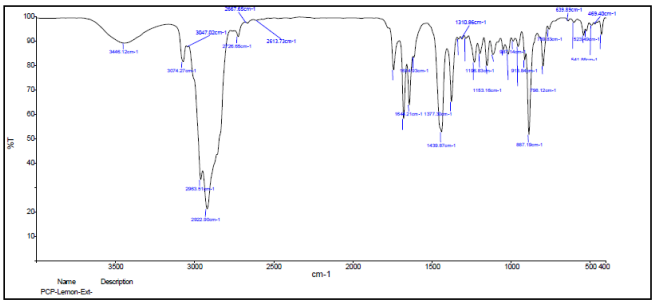

Fig. 24: FTIR Spectrum of Aqueous Extract of *Citrus limonum.* Risso.

Table 8: FTIR Interpretation of Aqueous Extract of *Citrus limonum.* Risso.

Wave number (cm^{-1})	Functional Group
3446.12	O-H stretching
3047	C-H Stretching
2963.51	C-H Stretching
1743.55	C-O Stretching
1614.93	C=C Stretching
1286.87	C-O Stretching
1153.16	C-O Stretching
1050	C-O Stretching

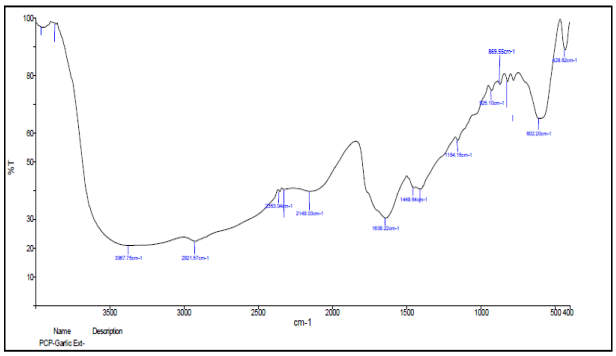

Fig. No. 25: FTIR Spectrum of Aqueous Extract of *Allium sativum*. Linn.

Table 9: FTIR Interpretation of Aqueous Extract of *Allium sativum*. Linn.

Wave number (cm^{-1})	Functional Group
3367.75	O-H stretching
2921.57	C-H Stretching
2320.15	N-H Stretching
2149.03	S-C≡N Stretching
1638.22	C=C Stretching
1449.54	C-H Bending
1403.38	S=0 Stretching

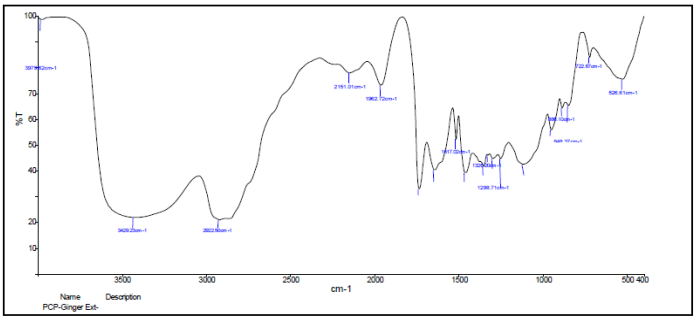

Fig. 26: FTIR Spectrum of Aqueous Extract of *Zingiberofficinale*. Roscoe.

Table 10: FTIR Interpretation of Aqueous Extract of *Zingiberofficinale*. Roscoe.

Wave number (cm^{-1})	Functional Group
3429.23	O-H stretching
2922.50	C-H Stretching
2151.01	S-C≡N Stretching
1962.72	C-H Bending
1735.59	C=O Stretching
1646.51	C=N Stretching
1326.20	O-H Bending

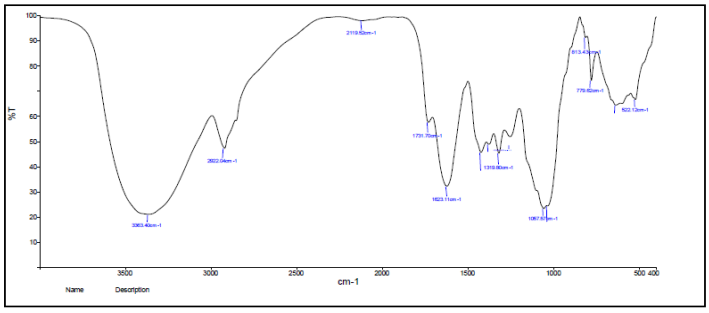

Fig. 27: FTIR Spectrum of *Aloe barbadensis.*

Table 11: FTIR Interpretation of *Aloe barbadensi.*

Wave number (cm^{-1})	Functional Group
3363.40	O-H stretching
2922.04	C-H Stretching
2119.52	C≡C Stretching
1731.70	C=O Bending
1623.11	C=C Stretching
1378.85	O-H Bending

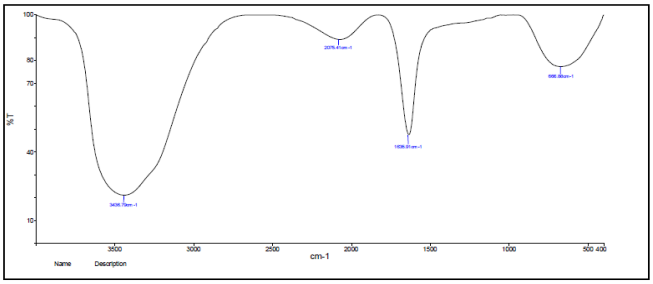

Fig. 28: FTIR Spectrum of Carbopol 934.

Table 12: FTIR Interpretation of Carbopol.

Wave number (cm^{-1})	Functional Group
3436.79	O-H stretching
2075.41	N=C=S Stretching
1635.91	C=C Stretching
666.88	C=C Bending

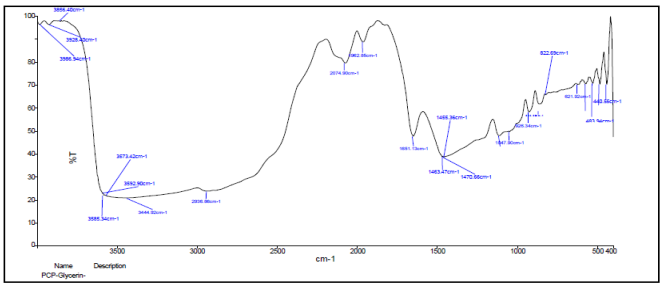

Fig. 29: FTIR Spectrum of Glycerin.

Table 13: FTIR Interpretation of Glycerin.

Wave number (cm^{-1})	Functional Group
3444.92	O-H stretching
2933.86	C-H Stretching
2074.90	N=C=S Stretching
1962.85	C-H Bending
1651.13	C=N Stretching
1111.44	C-O Stretching

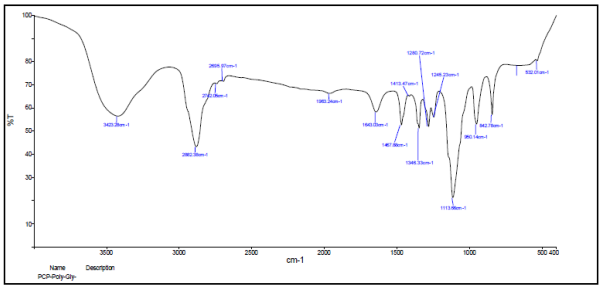

Fig. 30: FTIR Spectrum of Polyethylene glycol.

Table 14: FTIR Interpretation of Polyethylene glycol.

Wave number (cm^{-1})	Functional Group
3423.28	O-H stretching
2882.38	C-H Stretching
1643.24	C=N Stretching
1414.22	OH Bending
1346.33	O-H Stretching
1280.72	C-O Stretching
1245.23	C-N Stretching
1113.66	C-O Stretching

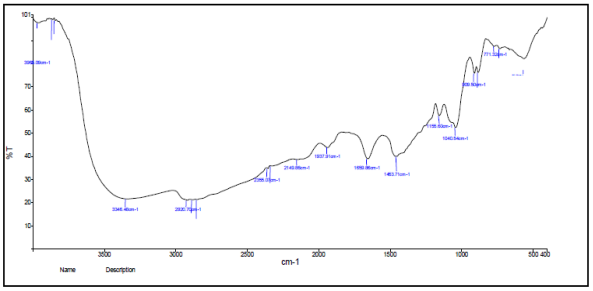

Fig. 31: FTIR Spectrum of Triethanolamine.

Table 15: FTIR Interpretation of Triethanolamine.

Wave number (cm^{-1})	Functional Group
3346.48	N-H stretching
2920.70	N-H Stretching
2882.62	C-H Stretching
2851.60	C-H Bending
2149.86	N=N=N Stretching
1659.86	C-H Bending
1453.71	C-H Bending

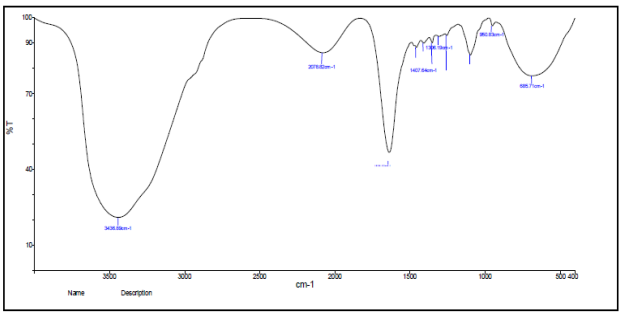

Fig. 32: FTIR Spectrum of Herbal Antidandruff Gel.

Table 16: FTIR Interpretation of Herbal Antidandruff Gel.

Wave number (cm^{-1})	Functional Group
3436.89	O-H stretching
2078.82	N=C=S Stretching
1639.52	C=C Stretching
1351.86	O-H Bending
1254.26	C-O Stretching
1306.19	C-N Stretching
1096.71	C-O Stretching

There are no extra peaks seen other than the normal peak in the spectra of the mixture of the extracts containing active constituents and excipients so there is no evidence of interaction with the drug and polymers and they are compatible with each other.

Physico chemical evaluation of Herbal Antidandruff Gel

Table 17: Physico chemical evaluation of Herbal Antidandruff Gel.

Formulations	Clarity	pH	Homogeneity	Spreadability (g.cm/sec)	Extrudability	Viscosity (cps)	% Drug Content
F1	Turbid	6.9	Not Good	10.08	+	8823	70.92
F2	Turbid	6.8	Not Good	12.89	+	8818	75.30
F3	Turbid	6.7	Not Good	12.27	+	8951	68.53
F4	Turbid	6.9	Not Good	13.86	+	8890	72.95
F5	Clear	7.1	Good	18.75	+ +	9632	79.82
F6	Clear	6.9	Good	20.55	+ +	9826	83.02
F7	Clear	7.0	Good	22.39	+ +	9142	78.92
F8	Clear	7.2	Good	18.07	++	9122	85.46

+ Satisfactory, ++ Excellent

Eight batches of Herbal Antidandruff Gel formulations were prepared by using Carbopol 940 and Carbopol 934 were subjected to various physicochemical evaluations. Based on the clarity, pH, homogeneity, spreadability, viscosity, percentage drug content and extrudability formulations F5, F6,F7,F8 were selected for further studies.

Optimized formula of herbal antidandruff gel

Table 18: Optimized formula of Herbal Antidandruff Gel.

S. No.	Ingredients	F5	F6	F7	F8
1.	*Emblica officinalis*	0.5ml	-	-	0.5ml
2.	*Citrus limonum*	-	0.5ml	-	0.5ml
3.	*Allium sativum*	-	-	0.5ml	0.5ml
4.	*Zingiber officinalis*	-	-	0.5ml	0.5ml
5.	*Aloe barbadensis*	-	-	0.5g	0.5g
6.	Carbopol 934	0.30g	0.30g	0.30g	0.30g
7.	Polyethylene Glycol	7g	7g	7g	7g
8.	Triethanolamine	0.6g	0.6	0.6g	0.6g
9.	Propyl Paraben	0.075g	0.075g	0.075g	0.075g
10.	Glycerine	3ml	3ml	3ml	3ml
11.	Water q.s	50ml	50ml	50ml	50ml

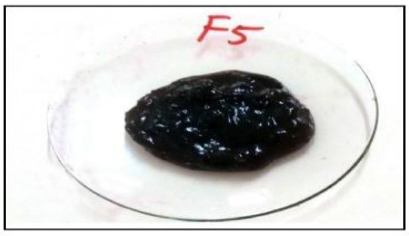

Fig. 33: Formulation F5

Fig. 34: Formulation F6

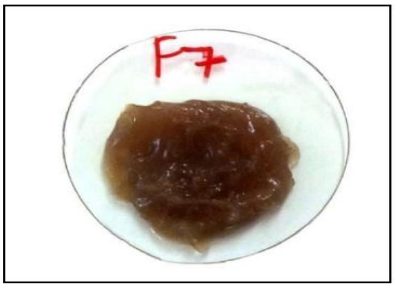

Fig. 35: Formulation F7

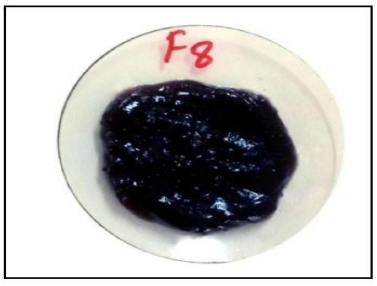

Fig. 36: Formulation F8

Physiochemical evaluation of best four formulations

Table 19: Clarity test.

Formulations	Clarity
F5	Clear
F6	Clear
F7	Clear
F8	Clear

Table 20: pH.

Formulations	pH
F5	7.1
F6	6.9
F7	7.0
F8	7.2

Table 21: Homogeneity test.

Formulations	Homogeneity
F5	Good
F6	Good
F7	Good
F8	Good

Table 22: Spreadability test.

Formulations	Spreadability
F5	18.75
F6	20.55
F7	22.39
F8	18.07

Table 23: Extrudability test.

Formulations	Extrudability
F5	79.82
F6	83.02
F7	78.92
F8	85.46

Table 24: Viscosity.

Formulations	Viscosity
F5	9632
F6	9826
F7	9142
F8	9122

Table 25: Drug content.

Formulations	Drug content
F5	79.82
F6	83.02
F7	78.92
F8	85.46

***In vitro* release studies**

Comparative *In vitro* release profile of F5, F6, F8A, F8B formulation

Table 26: Comparative *In vitro* release profile of F5, F6, F8A, F8B formulation.

S. No.	Time (in min)	% of release of F5 formulation	% of release of F6 formulation	% of release of F8A formulation	% of release of F8B formulation
1	0	0.000	0.000	0.000	0.000

2	5	4.577± 0.128	15.437±0.945	4.990±1.350	14.100±1.108
3	10	9.250 ±0.824	22.553±3.085	7.307±1.666	22.000±1.155
4	15	18.840±0.277	28.967±0.447	15.710±1.467	27.643±0.904
5	20	26.247±0.967	36.333± 1.480	22.937±0.998	34.763±1.644
6	25	32.093±0.340	40.780±0.435	29.873±2.830	43.093±1.322
7	30	38.820±1.688	48.220±1.446	38.500±1.267	50.817±0.981
8	60	47.573±0.637	58.173±1.615	45.843±1.554	57.570±1.445
9	90	58.000±0.386	68.533±0.996	56.667±1.576	68.977±1.520
10	120	73.100±1.097	79.627±1.873	71.773±1.356	77.413±1.987
11	180	81.000±1.528	84.333±1.564	83.000±1.528	84.977±0.989
12	240	66.333±1.333	74.667±1.987	63.333± 1.667	74.000±0.577
13	300	45.500±0.987	42.257±0.765	48.000± 1.528	43.000±1.528

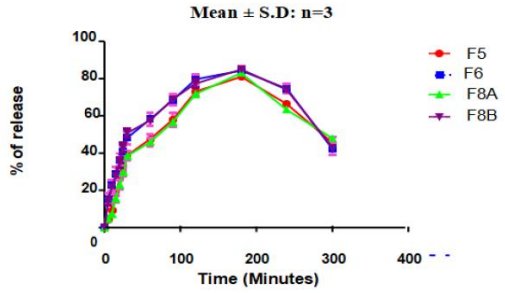

Fig.49. Comparative *in-vitro* release study of F5, F6, F8A, F8B

Diffusion kinetics

The optimized gel formulations of (F5, F6, F8A, F8B) were fitted to various kinetic equations to determine the mechanism of drug diffusion rate as indicated by maximum r^2 value.

Table 27: Diffusion kinetics of F5 formulation.

S. No.	Cummulative % release(Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	4.577	5	2.236	0.661	0.699	1.98
3	9.25	10	3.162	0.966	1	1.958
4	18.84	15	3.873	1.275	1.176	1.909
5	26.247	20	4.472	1.419	1.301	1.868
6	32.093	25	5	1.506	1.398	1.832
7	38.82	30	5.477	1.589	1.477	1.787
8	47.573	60	7.746	1.677	1.778	1.72
9	58	90	9.487	1.763	1.954	1.623
10	73.1	120	10.954	1.864	2.079	1.43
11	81	180	13.416	1.908	2.255	1.279
12	66.333	240	15.492	1.822	2.380	1.527
13	45.5	300	17.321	1.658	2.477	1.736

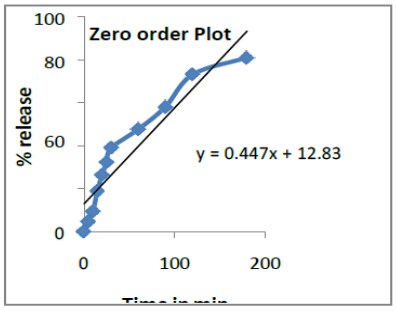

Fig. 37: Zero order kinetic plot F5.

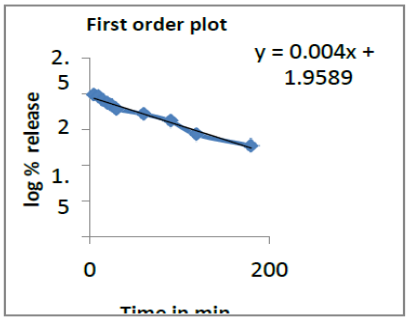

Fig. 38: First Order Kinetic Plot of F5.

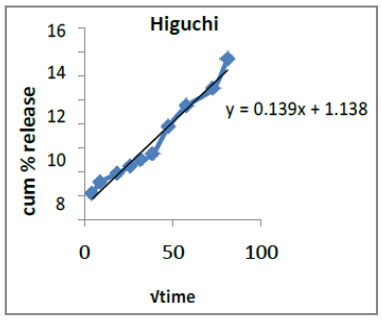

Fig. 39: Higuchi plot of F5.

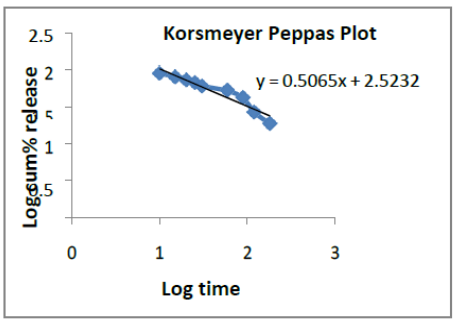

Fig. 40: Korsmeyer Peppas Plot of F5.

Table 28: Diffusion kinetics of F6 formulation.

S. No.	Cummulative % release(Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	15.43667	5	2.236	1.189	0.699	1.927
3	22.55333	10	3.162	1.353	1	1.889
4	28.96667	15	3.873	1.462	1.176	1.851
5	36.3333	20	4.472	1.560	1.301	1.804
6	40.78	25	5	1.610	1.398	1.772
7	48.22	30	5.477	1.683	1.477	1.714
8	58.173333	60	7.746	1.765	1.778	1.621
9	68.533333	90	9.487	1.836	1.954	1.498
10	79.626666	120	10.954	1.901	2.079	1.309
11	84.33334	180	13.416	1.926	2.255	1.195
12	74.66666	240	15.492	1.873	2.380	1.404
13	42.25667	300	17.321	1.626	2.477	1.762

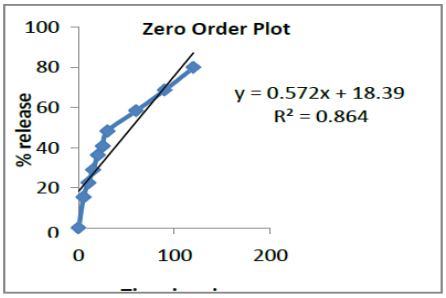

Fig. 42: Zero order plot of F6.

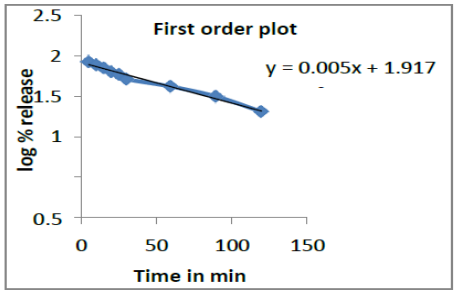

Fig. 43: First order plot of F6.

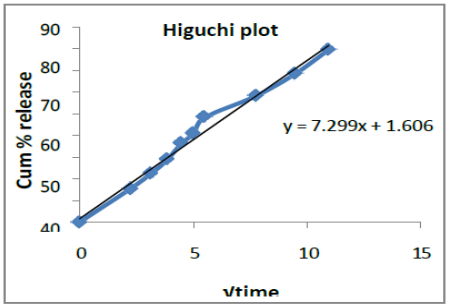

Fig. 44: Higuchi plot of F6.

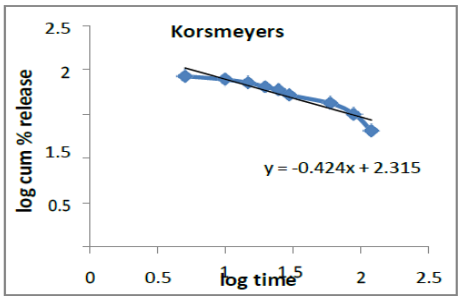

Fig. 45: Korsmeyer plot of F6.

Table 29: Diffusion kinetics of F8A formulation.

S. No	Cummulative % release(Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	4.99	5	2.236	0.698	0.699	1.978

3	7.306667	10	3.162	0.864	1	1.967
4	15.71	15	3.873	1.196	1.176	1.926
5	22.93667	20	4.472	1.361	1.301	1.887
6	29.87333	25	5	1.475	1.398	1.846
7	38.5	30	5.477	1.585	1.477	1.789
8	45.84333	60	7.746	1.661	1.778	1.734
9	56.66667	90	9.487	1.753	1.954	1.637
10	71.77333	120	10.954	1.856	2.079	1.451
11	83	180	13.416	1.919	2.255	1.230
12	63.33333	240	15.492	1.802	2.380	1.564
13	48	300	17.321	1.681	2.477	1.716

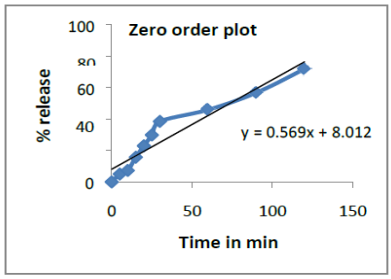

Fig. 46: Zero order plot of F8A.

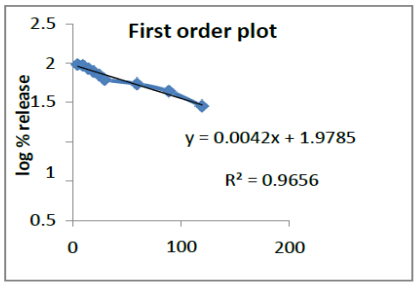

Fig. 47: First order plot of F8A.

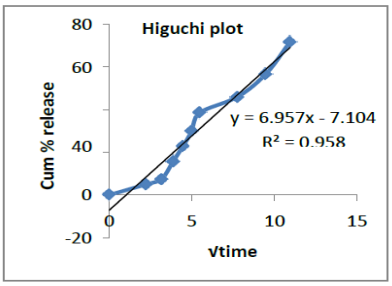

Fig. 48: Higuchi plot of F8A.

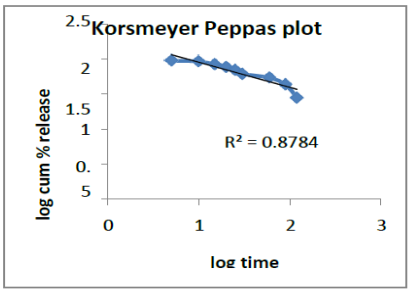

Fig. 49: Korsmeyer Peppas plot of F8A.

Table 30: Diffusion kinetics of F8B formulation.

S. No	Cummulative % release(Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	14.1	5	2.236	1.149	0.699	1.934
3	22	10	3.162	1.342	1	1.892
4	27.64333	15	3.873	1.442	1.176	1.859
5	34.76333	20	4.472	1.541	1.301	1.814
6	43.09333	25	5	1.634	1.398	1.755
7	50.81667	30	5.477	1.706	1.477	1.692
8	57.57	60	7.746	1.760	1.778	1.628
9	68.97667	90	9.487	1.839	1.954	1.492
10	77.41333	120	10.954	1.889	2.079	1.354
11	84.97667	180	13.416	1.929	2.255	1.177
12	74	240	15.492	1.869	2.380	1.415
13	43	300	17.321	1.633	2.477	1.756

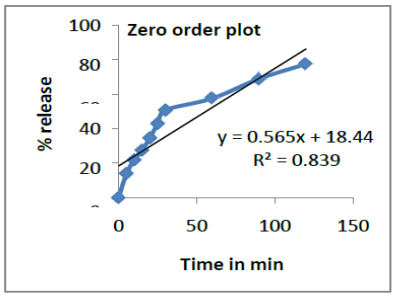

Fig. 50: Zero order plot of F8B.

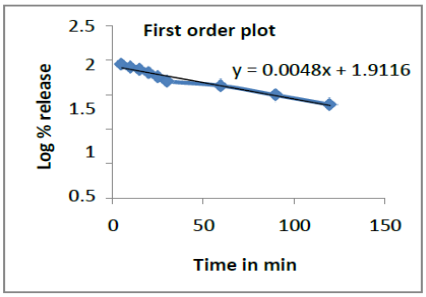

Fig. 51: First order plot of F8.

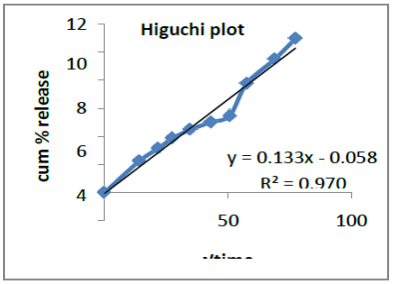

Fig. 52: Higuchi plot of F8B.

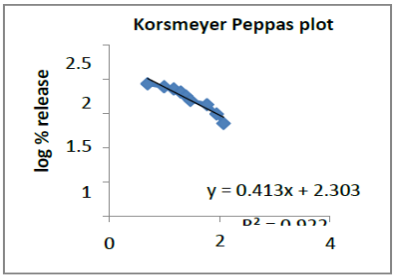

Fig. 53: Korsmeyers Peppas plot of F8B.

Table 31: Diffusion kinetics.

Formulationcode	Correlation coefficient(r^2)				„n"-Diffusion Exponent
	Zero order	First order	Higuchi	Korsmeyerpeppas	
F5	0.883	0.976	0.970	0.919	0.954
F6	0.864	0.980	0.985	0.896	0.875
F8A	0.914	0.965	0.958	0.878	0.963
F8B	0.839	0.963	0.970	0.922	0.960

Release kinetic study revealed that the F5,F6, F8A and F8B follows Zero order and non fickian diffusion model. So they were subjected to antimicrobial screening.

Screening of antimicrobial activity of optimized gel formulation

For Fungi

After 72h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no fungal growth around the patch. The figures are shown in Fig 54,55 and the results are shown in Table 31.

For Bacteria

After 24h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the patch. The Figures are shown in fig. 56, 57 and 58 and the results are shown in Table 31.

Fungi

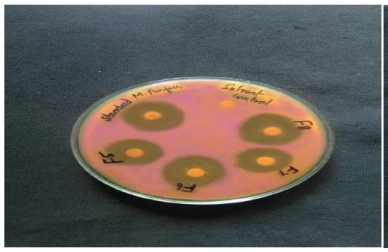

Fig. 54: *Malassezia furfur.*

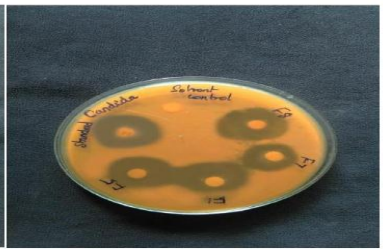

Fig. 55: *Candida albicans.*

Bacteria

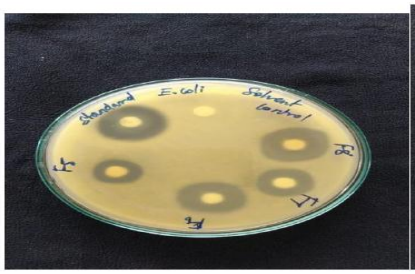

Fig. 56: *Escherichia coli.*

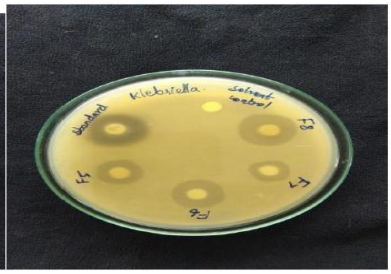

Fig. 57: *Klebsiella aerogenes.*

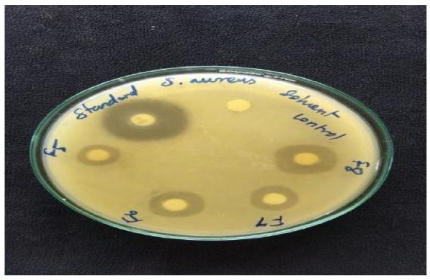

Fig. 58: *Staphylococcus aureus.*

Table 32: Screening of Antimicrobial activity.

S. No	Name of the Organism	Zone of Inhibition in mm					
		Sample				Solvent Control	Standard
		F5	F6	F7	F8		
1.	*Malassezia furfur* (MTCC 1765)	27	28	23	33	Nil	35
2.	*Candida albicans* (NCIM 3102)	25	27	20	29	Nil	32
3.	*Staphylococcus aureus* (NCIM 2079)	12	16	15	20	Nil	35
4.	*Escherichia coli* (NCIM 2065)	18	22	20	24	Nil	38
5.	*Klebsiella aerogenes* (NCIM 2098)	17	20	15	22	Nil	30

Stability study of Herbal Antidandruff Gel F8

Table 33: Stability study of F8.

S. No	Parameter	Observation						
		Initial	At the end of 1st month		At the end of 2nd month		At the end of 3rd month	
			RT	40±2°C & RH 70±5%	RT	40±2°C & RH 70±5%	RT	40±2°C & RH 70±5%
1.	Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
2.	pH	7.2	7.0	7.2	7.2	7.2	7.2	7.1
3.	Spreadibility	18.07	18.06	18.07	18.07	18.07	18.07	18.07
4.	Extrudability	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
5.	% drugcontent	85.46	85.46	85.44	85.46	85.46	85.46	85.46

The stability studies of Herbal Antidandruff Gel of formulation F8 was carried out for three months. During this period, the formulation were stable and showed no significant changes in visual appearance, pH, Spreadability, Extrudability, % drug content.

CONCLUSION

Eight batches of Herbal Antidandruff gel were formulated. All the formulated gels were subjected to Physiochemical evaluations such as Clearance, pH, Homogeneity, Spreadability, Extrudability, Viscosity, Drug content were evaluated. Based on the Physicochemical evaluations formulation F5, F6, F7 and

F8 were selected as the optimized gel formulation. The FTIR graphs of active constituents, excipients and formulations results showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there was noincompatibility between active constituents and excipients. For the above selected formulations, i*n-vitro* release profiles were performed. Base on the i*n-vitro* release profile it was found that release of active constituents from prepared gels followed first order kinetics. To confirm the release mechanism, the data of F5, F6, F7, F8 release were applied to Korsmeyer- peppas equation to find out the release exponent „n", which indicates the mechanism of drug diffusion from the gel formulation. Then they were subjected to Screening of antimicrobial activity. The stability study were performed for the selected formulation (F8) by both the technique as per the ICH guidelines. The gel was subjected to stability study at $40^{o}C\pm2^{o}C$ and 75±5% RH, samples were withdrawn on 1 month, 2 month, 3 month and analysed. The result shown that the product is stable for 3 months without change in physical changes. Since the antimicrobial studies has given encouraging results in enhancing the antidandruff activity of F8 formulation, it is concluded that the F8 Herbal antidandruff gel may be subjected to further *in-vivo* and clinical trials.

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