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PHYSICOCHEMICAL CHARACTERIZATION OF DIFFERENT POLY HERBAL FORMULATIONS

Megha Jain[1]*, Vinay Jain[2] and Priyanka Arjariya[3]

[1]Malwa Institute of Pharmacy, Gwalior.

[2]Department of Pharmacognosy, Shri Ram College of Pharmacy, Banmore, Morena.

[3]Department of Pharmaceutics, Shri Ram College of Pharmacy, Banmore, Morena.

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***Corresponding Author**
Megha Jain
Malwa Institute of Pharmacy, Gwalior.

ABSTRACT

Poly herbal cream is a semi solid formulation that is being developed for its therapeutic activity. The need for stability of the new formulation over the period of its shelf life remains a sacred factor. In the present study, creams were formulated by herbal extracts and they were evaluated. Selected part of different plants (*Triticum aestivum, Mentha piperita, Elaeocarpus ganitrus roxb, Evolvulus alsinoides linn.* and *Cyprus esculentus*) was dried and extracted using different suitable solvents and extraction methods. Quality evaluation of the product was assessed by using different evaluation methods. No change of the physical properties was observed; the pH was in a proper range (approximately pH 6). The formulations showed good spreadability, no evidence of phase separation and good consistency during this study period. It was found that the viscosity of the cream decreases when increasing the rate of shear so the viscosity of creams is inversely proportional to rate of shear (rpm). The creams were found to be stable during stability study (4 °C, 30 °C, 45 °C) for 45 days. From the present study it can be concluded that it is possible to develop creams containing poly herbal extracts. Poly herbal creates a synergy between ingredients that multiplies their effectiveness; as a result our formulas will work faster and be more effective.

KEYWORDS: Poly herbal cream, Triticum aestivum, Mentha piperita, Elaeocarpus ganitrus roxb, Evolvulus alsinoides linn., Cyprus esculentus Therapeutic activity, Stability, Shelf life.

INTRODUCTION

Nowadays people increasingly prefer alternative to conventional medicine. The reasons are it is safe and it works. While the allopathic medicine works well in the case of trauma and

emergency, it is much less effective when it comes to prevention, chronic disease and in addressing the mental, emotional and spiritual needs of an individual. These are precisely the areas where alternative medicine excels.[1]

Herbal medicine, as a major part of traditional medicine, has been used in medical practice since antiquity and is a common element of ayurvedic, homeopathic, and naturopathic medicine. World health organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures.[2] The global market of herbal drugs at present is approximately $ 600 billion.[3] Herbal cosmetics are the products in which herbs are used in crude or extract form.[4]

The plant parts used in cosmetic preparation should have varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrhatic, antikerolytic activity and antibacterial etc. Herbal products claim to have less side effects, commonly seen with products containing synthetic agents. The market research shows upward trend in the herbal trade with the herbal cosmetic industry playing a major role in fueling this worldwide demand for herbals.[5]

Most of the biologically active constituents of plants are polar or water soluble molecules. These poorly absorbed either due to their large molecular size which cannot be absorbed by passive diffusion or due to their poor lipid solubility thus limiting their ability to pass across the lipid rich biological membranes, resulting poor bioavailability when taken orally or applied topically.[6] Many approaches have been developed to improve the bioavailability, such as topical administration of the extract. Phospholipids based drug delivery systems have been found much hopeful and promising for the effective and efficacious herbal drug delivery. Creams are semisolid emulsion systems with opaque appearances, as contrasted with translucent ointments. Their consistency and rheological character depends on whether the emulsion is a water-in-oil or oil-in-water type and the nature of the solids in the internal phase.[7] Concern for the physical and chemical integrity of topical systems is no different from that of other dosage forms. However, there are some unique and germane dimensions to stability associated with semisolid systems.[8] Therefore, the present study was undertaken to develop cream formulation and to evaluate it with different parameters.

MATERIAL AND METHODS

Collection of Plants and Authentication: The Wheat grass from *T. aestivum* was grown in medicinal garden SRCP Banmore and collected on tenth day from growing. This was identified and authenticated by Dr. N.K. Pandey (Research officer Ayurveda) National Research institute for Ayurveda HRD, Gwalior (M.P) (Ref. no. 5-4/12-13/NRIASHRD/Tech/Survey/134). The leaf *Mentha piperita* collected from Jalone dist. U.P., identified and authenticated by Dr. N.K. Pandey (Research officer Ayurveda) National Research institute for Ayurveda HRD, Gwalior (M.P) (Ref. no. 5-4/12-13/NRIASHRD/Tech/Survey/134).

The leaf *Elaeocarpus ganitrus roxb.* was collected from Kankhal Shiva Mandir Haridwar, U.K. and identified and authenticated by Dr. Jitendra singh Pachaya (Asst. Professor Botany) Govt. PG College Alirajpur (M.P.) (Ref. no. 868). The tubers of *Cyprus esculentus* were collected from local market of Gwalior, identified and authenticated by Dr. Jitendra singh Pachaya (Asst. Professor Botany) Govt. PG College Alirajpur (M.P.) (Ref. no. 868). The Arial part of *Evolvulus alsinoides linn*. was collected from local market of Gwalior, identified and authenticated by Dr. Jitendra singh Pachaya (Asst. Professor Botany) Govt. PG College Alirajpur (M.P.) (Ref. no. 868).

Preparation of Extracts

Ethanolic Extract of *Triticum Aestivum, Evolvulus Alsinoides* and *Elaeocarpus Ganitrus Roxb:* Arial parts of the plant *T. aestivu E. alsinoides* and *Elaeocarpus Ganitrus Roxb.* were shade-dried and then powdered by using hand grinder and then sieved the powder by a sieve mesh size 0.12 for uniform particle size powder. Extract was prepared using soxhlet apparatus at a temperature of 40 to 50°C for a period of 3 days. The extracts were collected and distilled off on a water bath at 40°C atmospheric pressure and the last trace of the solvent was removed in *vacuo*. Extracts were stored in refrigerator at 4ºC until use. The residues were weighed and percentage yield were calculated.

Hydro Alcoholic Extract of *Cyprus Esculentus*

The tubers part of *C. esculentus* was shade-dried and then powdered by using hand grinder and then sieved the powder by a sieve mesh size 12 for uniform particle size powder. Coarse powder was extracted exhaustively in a soxhlet apparatus with mixture of Ethanol: water (7:3 ratios) for 72 h. The extracts were collected and distilled off on a water bath at atmospheric

pressure and the last trace of the solvent was removed *in vacuo*.[9] Extract was stored in refrigerator at 4ºC until use. The residue was weighed and percentage yield was calculated.

Prepration of Formulation

Non medicated Formulation

Prepared the cream without poly herbal extract using formula given in Table 1. Calculated quantities of beeswax, liquid paraffin and glyceryl monostearate were taken in one beaker and glycerol, water and cetyl alcohol in another. Both the beakers were maintained at 60ºC and all the ingredients were melted. Then oily phase was added to aqueous phase along with methyl paraben and propyl paraben and stirred continuously. As the temperature went down rose oil was added and mixed well until required consistency was obtained.

Preparation of mixture of poly herbal extracts (PHE)

Prepared three different concentrations mixture of PHE using five different plant extracts. Took different plants extracts with ratio given in table 2. Plant extracts and phosphatidyl choline were placed in a 100 ml. round-bottom flask and dissolved in 30 ml. of anhydrous ethanol. After ethanol was evaporated off, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant aqueous extract-phospholipid complex was transferred into a glass bottle, and stored in the room temperature.

Prepration of poly herbal Formulation (PHF)

Prepared the poly herbal Formulation using formula F-I. Calculated quantities of beeswax, liquid paraffin and glyceryl monostearate were taken in one beaker and glycerol, water and cetyl alcohol in another. Phytocomplex (2%, 4%, 6%) were dissolved in ethanol by sonication and maintained at 40ºC. Both the beakers were maintained at 60ºC and all the ingredients were melted. Then oily phase was added to aqueous phase along with methyl paraben and propyl paraben and stirred continuously. The phyto-complex was added to the mixture when the temperature dropped to 40ºC. As the temperature went down rose oil was added and mixed well until required consistency was obtained.[10]

Table. 1: Formula of Non Medicated Formulation.

S. No.	Ingredients	F- I
1.	Beeswax	12%
2.	Liquid paraffin	23%
3.	Glyceryl monostearate	12%
4.	Cetyl alcohol	11%
5.	Glycerol	5.5%
6.	Distilled water	36.5%
7.	Rose oil	q.s.

Table. 2: Ratio of different plant extract to form mixture.

S. No.	Code of Poly herbal extract	EEWG	MPO	EEEG	HAECE	EEEA
1.	PHE 2%	2	2	2	2	2
2.	PHE 4%	4	4	4	4	4
3.	PHE 6%	6	6	6	6	6

Table. 3: Formula of Poly Herbal Formulation of Different Concentrations.

S. No.	Name of Ingredients	PHF-I	PHF-II	PHF-III
1.	PHE	2%	4%	6%
2.	Beeswax	12%	12%	12%
3.	Liquid paraffin	23%	23%	23%
4.	Glyceryl monostearate	12%	12%	12%
5.	Cetyl alcohol	11%	11%	11%
6.	Glycerol	5.5%	5.5%	5.5%
7.	Distilled water	36.5%	36.5%	36.5%
8.	Rose oil	q.s.	q.s.	q.s.

Fig. 1: Phytocomplex Hair Cream in Different Concentrations.

Method of Evaluation of Formulations

1. Organoleptic Characters

By visual appearance, colour and odour was noted.

2. Presence of Foreign Particles/Grittiness

A pinch of product was rubbed on skin and then observed with magnifying glass; if it is free from rashes or eruption then it was considered as free from grittiness.[11]

3. Determination of pH

The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured using digital pH meter.[12]

4. Loss on Drying

Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^0C.[13]

Percentage loss on drying = 100 × (Wt – MW)/Wt

5. Globule Size Determination

1 ml. of cream was diluted to 10 ml. with glycerin. A few drops of this were transferred onto a glass slide and was focused in a microscope. By using eyepiece micrometer globule size was determined.[11]

6. Consistency or Hardness of Formulations

It was measured by Penetrometer. Three containers were filled carefully and completely, without forming air bubbles and stored at 25+0.5^{0}C for 24 hrs. Three samples were stored at 25 ± 0.5^{0}C and with shear for 5 min. Three samples were melted carefully and completely filled three containers, without forming air bubbles stored at 25±0.5^{0}C for 24 hrs. Test samples were placed on Penetrometer. Temperature of penetrating object was adjusted at 25±0.5^{0}C and position was also adjusted such that its tip just touches the surface of sample. Penetrating object was released for 5 sec. Depth of penetration was measured. Same was repeated with remaining containers.[14]

7. Viscosity

Viscosity of cream was determined by Brookefield viscometer. The viscosity measurements were done using Brookefield DV-II viscometer using LV-4 spindle. The developed formulation was poured into the adaptor of the viscometer and the angular velocity increased gradually from 0.5 to 20 rpm.[4]

8. Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into petridish. A hole bored at the centre and ointment was placed in it. The area of diffusion of the formulation was noted in different time intervals.[13]

9. Spreadability Studies

Two glass slides of standard dimensions were selected. The formulation whose spreadability had to be determined was placed over one of the slides. The other slide was placed on top of the formulations was sandwiched between the two slides across the length of 5 cm along the slide. 100 g weight was placed up on the upper slide so that the formulation between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan. A 30 g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0 cm and separate away from the lower slide under the direction of the weight was noted. The spreadability was then calculated from the following formula.[13]

Spreadability= MxL/T

M = weight tied to the upper slide (30g)

L =length of glass slide (5cm)

T =time taken in seconds.

10. Irritation Test

Marked an area of 1sq.cm on the dorsal surface of the shaved rabbit .The cream was applied on the specified area and time was noted. Irritancy, erythyma, edema were checked.[10]

11. Anti Microbial Test Using Well Diffusion Plate Method: A fungus (*Candida albicans*) was inoculated into a test tube containing three ml of distilled water (medium), using a flamed loop. Drops of fungus/water culture was mixed with the warm, melted, autoclaved PDA and poured into separate plates under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2h. When cooled, a well was made at the centre of the plate. The well was made by using a 6 mm cork borer or puncher that was sterilized with alcohol and flame.

Different formulations placed in well seprete plates. The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48h.[15]

12. Stability study

The different formulation (PHF-I, PHF-II, PHF-III) were stored at 4^0, 30^0 and 45^0C. The products were evaluated at interval of 15, 30 and 45 days for following parameters:

- Colour
- Odor
- Appearance
- pH
- Loss of Drying (%)
- Globule size (mm.)
- Hard ness (mm.)
- Viscosity (cps.) at 5 r.p.m.
- Spreadibility (g.cm/sec.)
- Diffusion (mm^2) 240 min.

These parameters performed according to previous followed methods.

RESULT AND DISCUSSION

Organoleptic characters of different formulations were examined by various organoleptic properties enlisted in Table. 4. All three formulations showed minor change in color, odor and appearance. All formulations were smooth and free from grittiness. The results of other evaluation parameters such as pH, Loss on drying, Globule size, Hardness or Consistency and Spreadability of different formulations are given in Table. 5. The pH of all formulations was found to be around 6 which is suitable for topical application because the pH of the skin is in the range of 4.5– 6. Hardness or consistency: Oil content was increased on increasing the concentration of PHE which may be responsible for decreasing the consistency of formulations in the following order: PHF-I (200 mm)> PHF-II (235 mm) > PHF-III (248 mm). Spreadability was found to be decreased on increasing consistency of formulations from PHF-I to PHF-III. Viscosity of formulations was measured at different rates of shear. On increasing the rate of shear viscosity was found to be decreased. At constant r.p.m. (20) viscosities of different formulations were found in the following order: PHF-I (672 cps)> PHF-II> (654 cps) > PHF-III (614 cps). Highest loss on drying was found in case of PHF III which might be due its highest volatile content. It is evident from the result that as the

concentration of poly herbal extract was increased; oil content of formulation was increased resulting in decreasing viscosity of formulations. Viscosity measurement is shown in Table: 6, and Globule diameter of formulations was found in the following order: PHF-I (4.48 mm)> PHF-II (4.42 mm) > PHF-III (4.38 mm). On performing the diffusion study of different formulations results were obtained in the following order: PHF-III (1.5 cm.) > PHF-II (0.9 cm.)> PHF-I (0.6 cm.). As the oil content of cream increased rate of diffusion was found to be increased. It might be due to the fact that on increasing the oil content viscosity is decreased, resulting in decreasing resistance for the diffusion of drug. Another reason of highest rate of diffusion of formulation PHF-III might be smallest globule diameter resulting in highest surface area. The result of Diffusion study shows in Table: 7. It is evident from the result of zone of inhibition that formulation PHF III has highest resistant to microbial growth. All three formulations was non irritant which is shows in Table: 8, so it safe to topical application. Results of stability studies showed that no significant changes were observed in the parameters which were evaluated for stability studies.

Table. 4: Organoleptic Properties of Different PHF.

S. No.	Organoleptic Propeties	PHF-I	PHF-II	PHF-III
1.	**State**	Semisolid	Semisolid	Semisolid
2.	**Colour**	Light green	Dark Green	Greenish- black
3.	**Odour**	Characteristic	Characteristic	Characteristic
4.	**Appearance**	Homogenous	Homogenous	Homogenous

Table. 5: Result of Different Evaluation of All Three Formulations.

S. No.	Evaluation Parameters	PHF-I	PHF-II	PHF-III
1.	**pH**	6.30	6.50	6.80
2.	**Loss on Drying (w/w)**	37.0%	41.0%	43.0%
3.	**Globule Size (mm.)**	4.48	4.42	4.38
4.	**Hardness or Consistancy (mm.)**	200	235	248
5.	**Spreadability (g.cm./sec.)**	9.37	11.53	13.63
6.	**Antimicrobial Activity (mm^2)**	65.0	71.0	79.0

Table. 6: Viscosity of Different Formulation.

r.p.m.	Viscosity (cps.)		
	PHF-I	PHF-II	PHF-III
20	672	654	614
10	921	892	812
5	1582	1489	1314
1	3185	3085	2965
0.5	6752	6581	6346

Table. 7: Diffusion of Different Formulations.

Formulation code	Diffusion (cm.)		
	60 min.	120 min.	240 min.
PHF-I	0.1	0.38	0.6
PHF-II	0.2	0.7	0.9
PHF-III	0.35	1.0	1.5

Table. 8: Skin Irritation Test on Rabbits.

Formulation code	Erythema Score		Edema Score	
	Non abraded	Abraded	Non abraded	abraded
PHF I	0	0	0	0
PHF II	0	0	0	0
PHF III	0	0	0	0

Table. 9: Stability Study of Different Formulations at 4^0C.

S. No.	Parameters	PHF-I			PHF-II			PHF-III		
		15	30	45	15	30	45	15	30	45
1.	Colour	-	-	-	-	-	-	-	-	-
2.	Odor	-	-	-	-	-	-	-	-	-
3.	Appearance	-	-	-	-	-	-	-	-	-
4.	pH	6.30	6.30	6.30	6.40	6.50	6.40	6.80	6.80	6.80
5.	Loss of Drying (%)	37.0	37.0	37.0	41.0	41.0	41.0	43.0	43.0	43.0
6.	Globule size (mm.)	4.48	4.48	4.48	4.42	4.42	4.43	4.38	4.38	4.38
7.	Hard ness (mm.)	200	200	197	335	333	335	248	248	240
8.	Viscosity (cps.) at 5 r.p.m.	1582	1582	1582	1489	1489	1487	1314	1314	1314
9.	Diffusion (mm^2) 240 min.	0.60	0.60	0.60	0.92	0.90	0.90	1.50	1.60	1.55
10.	Spreadibility (g.cm/sec.)	9.37	9.37	9.37	11.5	11.5	11.5	13.63	13.63	13.63

(-) = No Change

Table. 10: Stability Study of Different Formulations at 30^0C.

S. No.	Parameters	PHF-I			PHF-II			PHF-III		
		15	30	45	15	30	45	15	30	45
1.	Colour	-	-	-	-	-	-	-	-	-
2.	Odor	-	-	-	-	-	-	-	-	-
3.	Appearance	-	-	-	-	-	-	-	-	-
4.	pH	6.30	6.30	6.30	6.50	6.50	6.50	6.80	6.80	6.80
5.	Loss of Drying (%)	37.0	37.0	37.0	41.0	41.0	41.0	43.0	43.0	43.0
6.	Globule size (mm.)	4.47	4.48	4.47	4.42	4.42	4.40	4.38	4.37	4.37
7.	Hard ness (mm.)	200	200	202	335	333	330	248	248	249
8.	Viscosity (cps.) at 5 r.p.m.	1582	1582	1580	1489	1487	1487	1314	1312	1312
9.	Diffusion (mm^2) 240 min.	0.60	0.60	0.60	0.90	0.91	0.90	1.50	1.50	1.55
10.	Spreadibility (g.cm/sec.)	9.35	9.35	9.35	11.52	11.50	11.50	13.63	13.63	13.63

(-) = No Change

Table. 11: Stability Study of Different Formulations at 45^0C.

S. No.	Parameters	PHF-I			PHF-II			PHF-III		
		15	30	45	15	30	45	15	30	45
1.	**Colour**	-	-	-	-	-	-	-	-	-
2.	**Odor**	-	-	-	-	-	-	-	-	-
3.	**Appearance**	-	-	-	-	-	-	-	-	-
4.	**pH**	6.30	6.30	6.30	6.50	6.50	6.60	6.80	6.70	6.80
5.	**Loss of Drying (%)**	37.0	37.0	37.0	41.1	40.9	40.8	43.0	42.8	42.8
6.	**Globule size (mm.)**	4.48	4.47	4.46	4.42	4.46	4.46	4.37	4.36	4.36
7.	**Hard ness (mm.)**	202	204	205	335	337	337	4.47	4.46	4.46
8.	**Viscosity (cps.) at 5 r.p.m.**	1576	1570	1567	1485	1480	1578	1310	1308	1305
9.	**Diffusion (mm^2) 240 min.**	0.60	0.60	0.55	0.90	0.90	0.80	1.50	1.52	1.50
10.	**Spreadibility (g.cm/sec.)**	9.34	9.26	9.29	11.49	11.48	11.42	13.58	13.55	13.55

(-) = No Change.

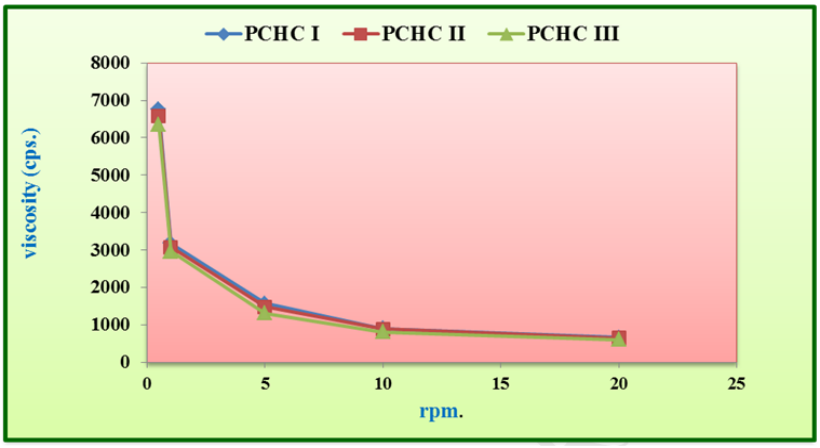
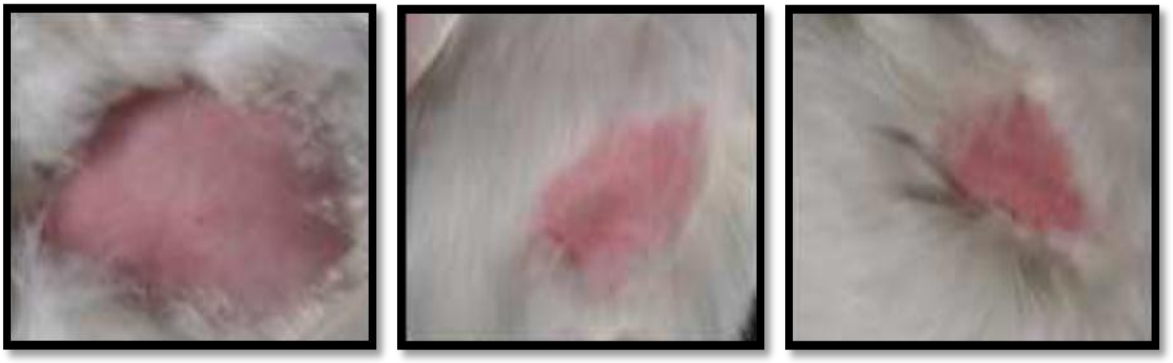
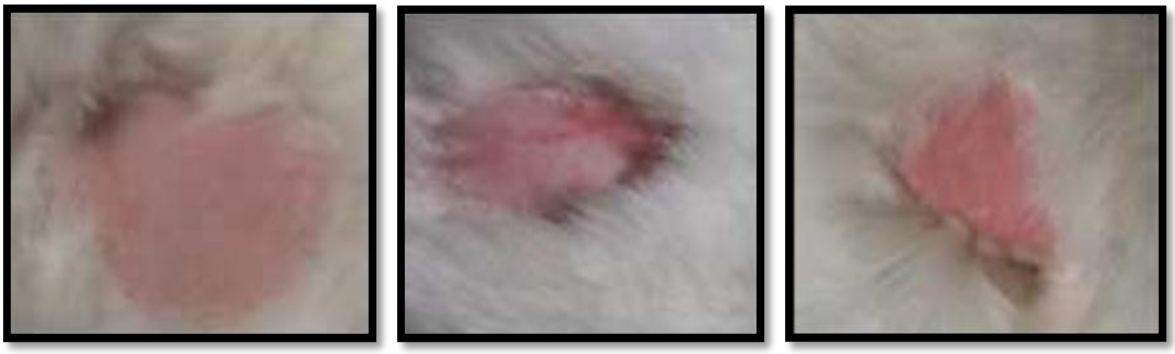


Fig. 2: Viscosity of Different Formulations.



(A_1) **(A_2)** **(A_3)**



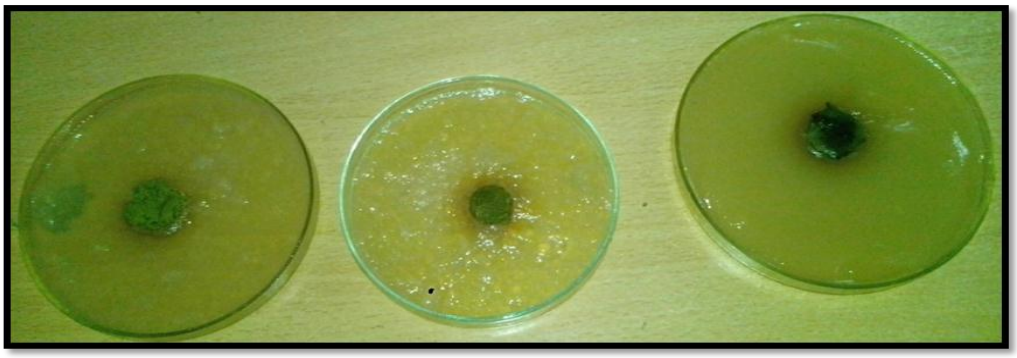
(B_1) **(B_2)** **(B_3)**

(B_1) = PHF-I Treated Group

(B_2) = PHF-II Treated Group

(B_3) = PHF-III Treated Group

Fig. 3: Skin Irritation Test on Rabbits.



PHF 2% **PHF 4%** **PHF 6%**

Fig. 4: Diffusion Study of Formulated Cream.

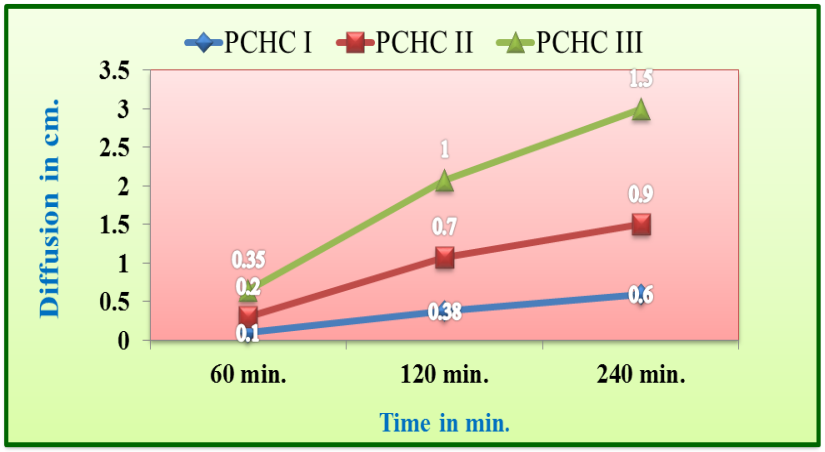


Fig. 5: Diffusion of Different Formulations.

CONCLUSION

Three poly herbal formulations of different concentration using mixture of five different plant extracts were prepared. Three different formulations (PHF I, PHF II and PHF III) were obtained. These were evaluated for different parameters such as globule diameter, loss on drying, pH, consistency, spreadability, viscosity, diffusion, antimicrobial activity and skin irritation test. Formulation PHF III was found to be best with respect to all parameters. Stability study was also performed for all formulations. No appreciable changes were found in any parameter, indicated the all formulations to be stable.

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