FORMULATION AND EVALUATION OF HERBAL ANTI-ACNE EMULGEL OF BERBERIS ARISTATA

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ABSTRACT: Acne is commonly known as multifactorial chronic inflammatory disease of pilosebaceous units. Bacteria that contributes to causing acne are Propionibacterium acnes and Staphylococcus epidermidis Acne occurs at any age mainly in adolescents. Dermatologists are still finding successful treatments for acne. In the market, there are varierty of anti-acne topical preparation are available, such as topical creams, gels & patches. The herbal formulation has various advantages over synthetic formulation. So herbal drug Berberis aristata was found to be an efficacious and cost-effective anti-acne drug as compared to other drugs used in the treatment of acne. Therefore this drug was selected to formulate an anti-acne emulgel. In this present research work the Propolis used as a novel excipient have activities like anti-acne, anti-oxidant, and anti-inflammatory. Propolis has been used as an anti-oxidant in the formulation but it also shows the additional effect with the activity of Berberis Aristata. The present work shows the formulation of Berberis aristata emulgel by performing the 3 formulation development approaches. The optimized batch is selected based on its appearance, consistency, homogeneity, and drug release.

KEYWORD: Acne, Emulgel, Propolis, Berberis aristata, Herbal

Introduction [1-6]

Over the last decades, the treatment of ailments has been accomplished by the administration of a drug to the human body through oral, rectal, sublingual, or parental routes. The topical drug delivery system is used where this system fails to administer the drug The main advantage of the topical delivery system is to bypass first-pass metabolism. Topical drug delivery can be defined as a way to deliver medication that is applied to the skin to treat various ailments.

Dermatological products containing drugs applied to the skin are diverse in formulation and range in consistency from solid to liquid but semisolid products are the most popular. In cosmetics and pharmaceutical preparation the use of gel has been increased. As compared with creams and ointments the gel formulation delivers faster drug release. Regardless of the many advantages of gels difficulty in hydrophobic drug delivery is a major limitation so to overcome this limitation emulgel is prepared and with their use, even a hydrophobic drug can enjoy the unique properties of gels. Emulgels are a combined form of emulsion and gels, water-in-oil and oil-in-water types of emulsion mixed in gel to form emulgel. Direct (oil-in-water) system is used to entrap lipophilic drugs whereas hydrophilic drugs are enclosed/entrapped in a reverse system (water-in-oil). Emulsions have a high ability to penetrate the skin and are also easily washed off whenever pertinent. Emulgels for skin have several properties such as being easily spreadable, easily removable, greaseless, water-soluble, and thixotropic.

The skin is perhaps the most endangered part of our body. It is customary fact that gradually exposure of human skin to the external environment leads to many problems such as sunburn marks, acne, and pigmentation. Acne is a common disorder experienced in the age group of 15-25 years due to the high level of sebum production continued by the attack of *Propionibacterium acnes*. The proposed research work is designed to study the impact of herbal emulgel to combat acne. The work emphasizes the topical treatment of acne, based on reported scientific data on emulgel prepared from the different herbal extracts. The treatment modalities for acne are usually directed at lowering the *P. acnes* population, producing an anti-inflammatory effect, and decreasing the sebaceous gland activity. Usually, to treat acne antibiotics and hormones are applied, for various years. However, these agents often coexist with drug resistance and severe side effects.

In this state affairs, ethanolic extracts of propolis and root of B. aristata have been screened for the aforementioned anti-acne activity. Propolis is a novel excipient used in the formulation. It is a natural resinous mixture produced by honeybees. There are two types of topical delivery products available. They are external and internal. As their names indicate, the internal products are applied orally, vaginally, and rectally and external products are applied by spreading or spraying.

FIG 1: Propolis and Berberis aristata

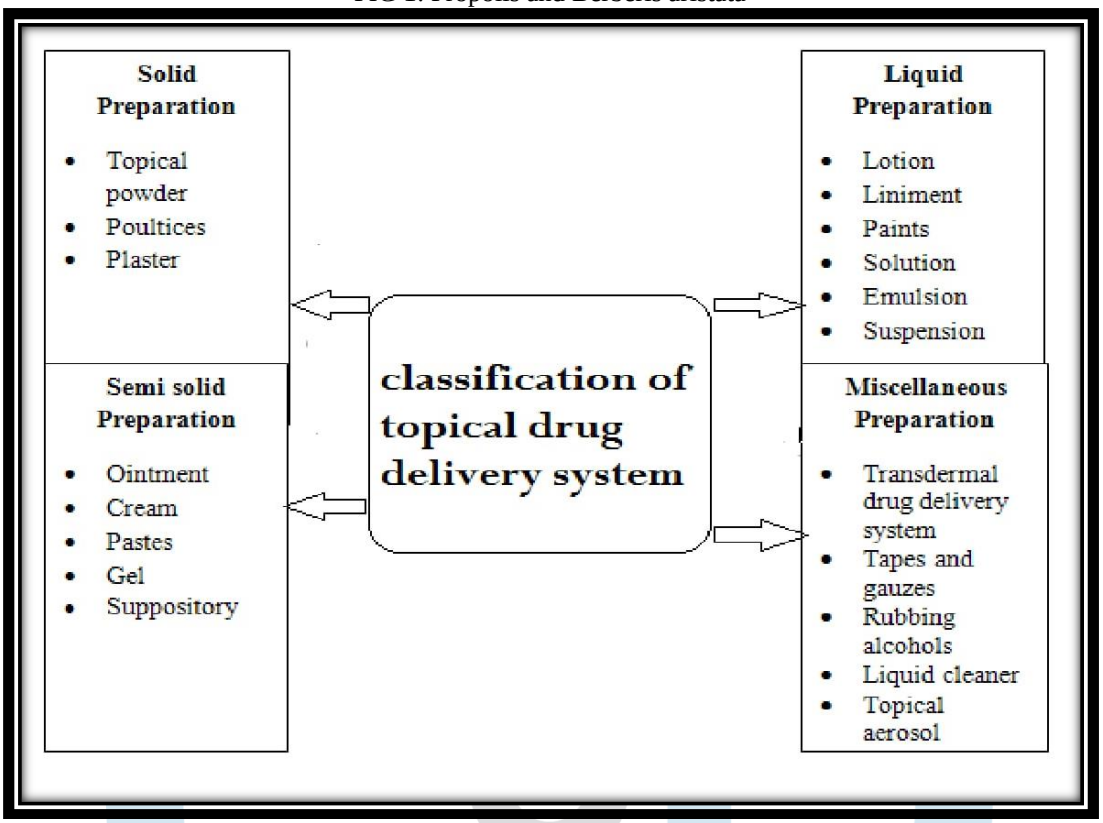

FIG 2: CLASSIFICATION OF TOPICAL DRUG DELIVERY SYSTEM

The absorption of the drug is affected by some factors through every route such as skin pH, hydration, partition coefficient, skin thickness, and molecular weight. The topical delivery system has many advantages and disadvantages. The main advantages are gastrointestinal incompatibility and avoidance of first-pass metabolism. Almost all topical preparation is applied to the skin. They penetrate through the skin and give the action on the target site. The skin is the largest organ in the body and has a surface of about 1.5 to 2 m2 in adults and involves nails, hair, and glands. The skin I composed of two main layers: The epidermis and the dermis. Between the skin and underlying structures the subcutaneous layer is present which consists of areolar and adipose (fat) tissue.

A) THE EPIDERMIS

The epidermis is the most superficial layer of skin and is composed of stratified keratinized squamous epithelium, which has different thicknesses in several parts of the body. The cells on the surface are flat, thin, non-nucleated, and squamous in which the cytoplasm has been replaced by the fibrous protein keratin. These are various strata of cells in the epidermis which extend from the deepest germinative layer to the most superficial stratum corneum. The complete replacement of the epidermis takes about one month.

B) THE DERMIS

The dermis is elastic and tough. It is established from the matrix contains collagen fibers entwined with elastic fibers and connective tissue. Macrophages, fibroblast, and mast cells are found in the dermis.

The structure of the dermis includes:

- Blood vessels
- Lymph vessels
- Sensory nerve endings
- Sweat gland and their ducts
- Hairs, arrector pili muscles, and sebaceous gland

C) SUBCUTANEOUS CONNECTIVE TISSUE

The hypodermis is not considered as a true part of structural connective tissue. It is composed of loss textured, white, fibrous connective tissue containing blood and lymph vessels, secretory pores of the sweat gland, and cutaneous nerves. Most investigators consider the drug is permeating through the skin and enter the circulatory system before reaching the hypodermis although the fatty tissue could serve as a depot of the drug

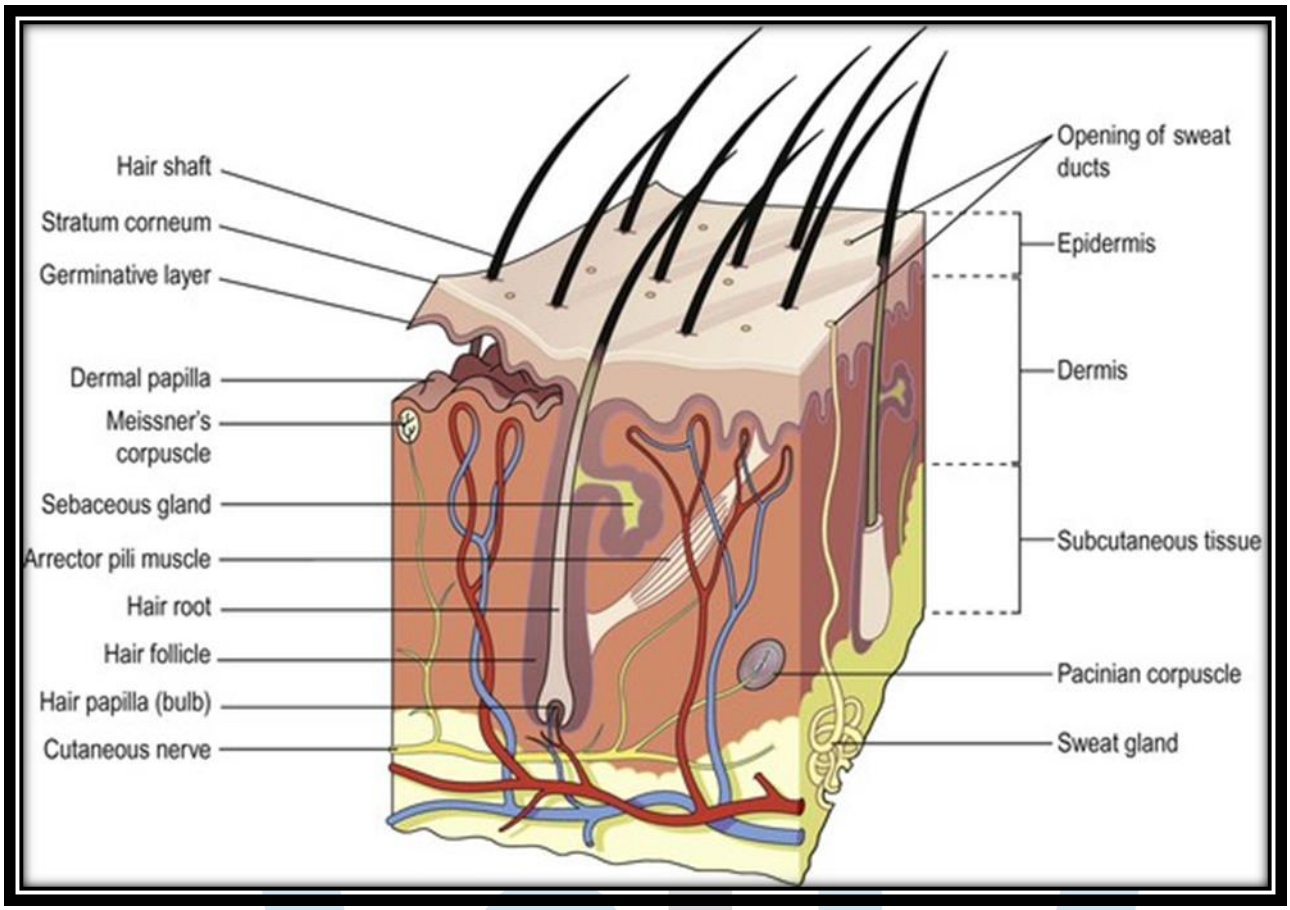

FIG NO 3: THE SKIN

➢ **DRUG DELIVERY ACROSS THE SKIN**

The skin established a relatively waterproof layer that protects the deeper and more delicate structures Blood vessels are distributed profusely beneath the skin. Especially important is a continuous venous plexus that is provided by an inflow of blood from the skin capillaries. Unique exposure to dermatological pharmacology is the direct accessibility of the skin as a target organ for diagnosis and treatment. The skin acts as a two-way barrier to prevent neither absorption nor water and electrolyte. Transcellular, intercellular and follicular are the three mechanisms of topical drug absorption. The pilosebaceous route is the most usual route of drug delivery. Mainly drugs are absorbed by passive diffusion, and they can enter the bloodstream if the drug can penetrate the stratum corneum. The reservoir and matrix are the two ideas in design of the transdermal drug delivery system. Both include the diffusion of drugs through the skin. For delivery of low permeability coefficient macromolecules, the improvement effects required and the capability of chemical enhancer tolerated by the skin. The addition of permeation enhancers such as fatty acids, esters, surfactants, and alcohols exerts their action by a temporary alteration of barrier properties of the stratum corneum by including enhancing solubility, fluidizing the crystalline structure of the stratum corneum, partitioning the stratum corneum and dissolution of stratum lipids enhances the drug flux. The gels and creams are used to deliver medication and infection-fighting drugs to an affected area of the body.

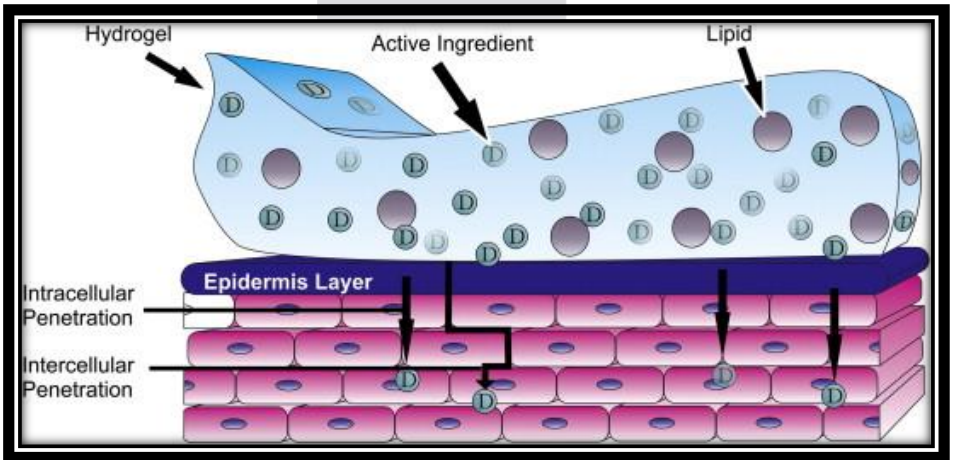

FIG 4: Mechanism of permeation of emulgel

➢ **RATIONALE OF EMULGEL AS A TOPICAL DRUG DELIVERY SYSTEM**

Cream, lotion, and ointments are usually used as topical agents and have various disadvantages. They have a lower spreading coefficient and need to be applied with rubbing. When they are applied to the skin causes uneasiness and exhibit problem of stability. Due to these factors use of transparent gels has expanded both in pharmaceutical and cosmetics preparation. Many medicinal products applied to skin or mucus membrane enhance or restore a fundamental function of the skin or pharmacologically alter an action in the underlying tissue.

- **FACTORS AFFECTING TOPICAL ABSORPTION OF DRUG**

A. Physiological factors

- Skin thickness
- Lipid content
- Skin pH
- The density of sweat gland
- Inflammation of skin
- Blood flow
- Type of skin
- Density of hair follicle

B. Physicochemical Factors

- Effect of vehicles
- Partition coefficient
- Degree of ionization
- Molecular weight (<500 Dalton)

C. Vehicle

- Solubility / polarity
- Concentration
- Volatility
- Distribution in stratum corneum
- Excipients
- Penetration enhancer
- pH

D. site of application

- skin area dose
- total skin area in contact with vehicle
- duration of exposure

MATERIAL AND METHOD[7-8]

1) **Material**: -

- Dried stem of Berberis aristata powder purchased from Chakrapani Ayurveda Clinic & Research Center, 8, Diamond Hill, Shanti Path, Behind Birla Temple, Jaipur (Raj), India. Mob: -0141-2624003
- Propolis which natural resinous mixture produced by honeybees, purchased from Amsar Pvt. Ltd. Kila Maidan Indore, 47 fort Industrial Estate Kila maidan, Indoor - 452006 (Laxmibai Nagar).
- All excipients Carbopol 934, parabens, tween 80, span 80, trim ethylene propylene glycol are procured from the laboratory of Modern College of Pharmacy (for ladies) Moshi Pune. All excipients are of analytical grade.
- Bacterial culture for microbial assay (Propionibacterium acne) purchase from CSIR institute of microbial Technology Sector 39-A, Chandigarh 160036 India.

Excipient were selected based on compatability of drug and excipient

Table 1: Excipients and their uses

SR NO.	EXCIPIENTS	CATEGORY
1	Propolis	Antioxidant , anti-inflammatory, antimicrobial
2	Carbopol 934	Effective as thickening agent for example emulsion, suspensions, sustained release formulations, transdermals,and topicals
3	Coconut Oil	Occlusive / oil phase
4	Span 80	emulsifier
5	Tween 80	emulsifier
6	Propylene Glycol	Co-surfactant/ penetration enhancer
7	Methyl Paraben	preservative
8	Propylparaben	preservative
9	Triethanolamine	PH adjuster
10	Water	vehicle

- **PREFORMULATION**

Table 2: Preformulation

SR No	Parameter	Observation
1	Solubility	Soluble in alcohol
2	Melting Point	145-146^{0}c
3	pH	3.4

- **COMPATIBILITY**

For Compatibility study, we kept combination of drug + propolis & drug+ carbopol for 30 days to check any colour change

Table 3: Compatibility Study

SR NO	Combination	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day30
1	Drug + Propolis	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change
2	Drug + Carbopol	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change	No Colour Change

2) Method: -

A] a) Preparation of Ethanolic extract of Berberis aristata and Propolis

1) Ethanolic extract of Berberis aristata dried stem powder 5gm macerated with 100ml ethanol in a closed flask for up to 24hr with shaking in-between frequently during six hours and stands for up to forty-two hrs. And then filter and take filtrate as ethanolic extract of Berberis aristata.

2) Ethanolic extract of propolis prepared by adding 2gm of propolis to 25ml of 10-95% ethanol.

b) Preparation of gel:

Prepare gel by adding gelling agent in the water with constant stirring with the help of magnetic stirrer at 1500 rpm.

c) Preparation of emulsion:

a) Preparation of oil phase

Prepare by adding span 80 as oil soluble emulsifying agent that is coconut oil

b) Preparation of water phase

Add Berberis aristata and propolis extract in water phase and add tween 80 as water soluble emulsifying agent. Then add preservatives methyl & propyl paraben with propylene for its solubility.

Separately at to 80c and then oil phase adds in aq. Phase with constant stirring till cool at room temperature.

d) Preparation of emulgel:

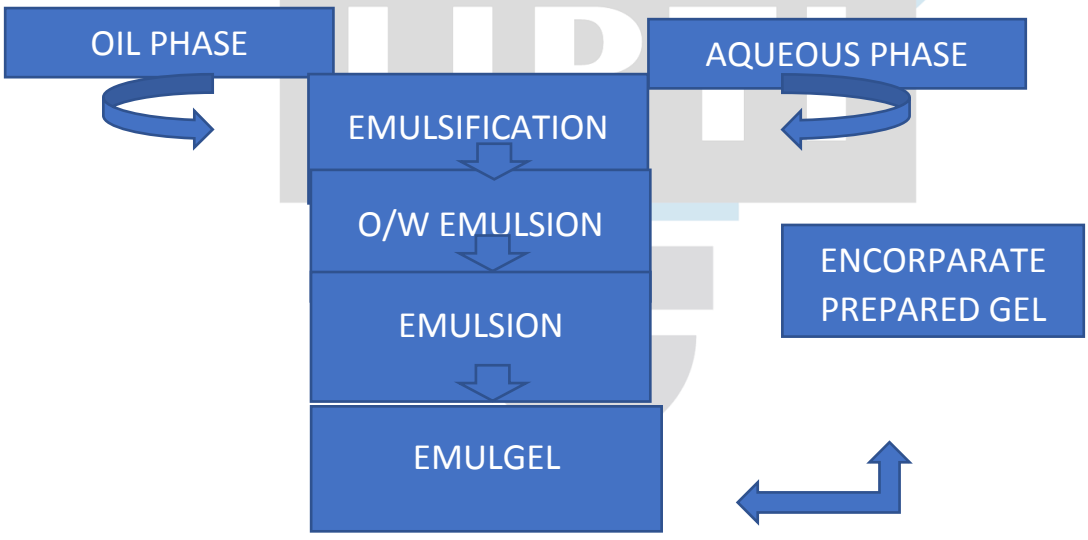

FIG 5: Process of emulgel formulation

B] Formulation Development

a) Experimental batches-

1) **Preliminary formulation:**

-In this batch dummy emulgel is prepared without using active ingredient. Here, liquid paraffin used as oil phase.

Table No 4: Preliminary Batch

Ingredients	F
Berberis aristata	-

propolis	3ml
Liquid Paraffin	7.5ml
Carbopol 934	1gm
Span 80	0.5ml
Tween 80	1.5ml
Propylene glycol	3.5ml
Methyl paraben	0.03gm
Propyl paraben	0.01gm
Triethanolamine	q.s
Water	q.s

Observation:
Due to use of liquid paraffin, there is no homogenecity in preparation and there is not good odor to formulation.

Fig 6: Preliminary Formulation

2) **Formulation development using different approaches**
a) Approach 1 -Batch 1 (F1) has formulated by using coconut oil instead of liquid paraffin as oil phase and by reducing the concentration of carbopol from 1 gm. to 0.5 gm.

Table No 5 – F1 Batch

Ingredients	**F1**
Berberis aristata	6ml
propolis	3ml
Coconut oil	43ml
Carbopol 934	0.5gm
Span 80	0.5ml
Tween 80	1.5ml
Propylene glycol	3.5ml
Methyl paraben	0.03gm
Propyl paraben	0.01gm
Triethanolamine	q.s
Water	q.s

Observation:
Homogenecity is achieved but consistency is not achieved. Also phase separation was observed.
b) Approach 2 -
Batch 2 (F2) has formulated by increasing the amount of oil phase from 43ml to 46ml and by changing the concentration of carbopol from 0.5 gm. to 1gm

Table No 6 - F2 Batch

Ingredients	**F2**
Berberis aristata	6 ml
propolis	3ml
Coconut oil	46ml
Carbopol 934	1gm
Span 80	0.5ml
Tween 80	1.5ml
Propylene glycol	3.5ml
Methyl paraben	0.03gm
Propyl paraben	0.01gm
Triethanolamine	q.s
Water	q.s

Observation:

Homogenecity and consistency is achieved and no phase separation.

b) Approach 3 -

Batch 3 (F3) has formulated by changing the concentration of carbopol from 1gm to 1.5gm

Table No 7- F3 Batch

Ingredients	**F3**
Berberis aristata	6 ml
Propolis	3ml
Coconut oil	46ml
Carbopol 934	1.5gm
Span 80	1ml
Tween 80	1ml
Propylene glycol	3.5ml
Methyl paraben	0.03gm
Propyl paraben	0.01gm
Triethanolamine	q.s
Water	q.s

Observation:

Homogenecity not achieved.

b) Optimized batch:

Table No 8- Optimized Batch

Ingredients	**F2**
Berberis aristata	6 ml
Propolis	3ml
Coconut oil	46ml
Carbopol 934	1gm
Span 80	0.5ml
Tween 80	1.5ml
Propylene glycol	3.5ml
Methyl paraben	0.03gm
Propyl paraben	0.01gm
Triethanolamine	q.s
Water	q.s

FIG 7: Optimized Batch

Evaluation Studies:

1) Physical parameters-

Emulsion formulation was inspected for its color, odor, homogeneity, phase separation, and grittiness.

2) pH-

Prepare 1% aqueous solution of emulgel and measure its pH by using digital pH meter. The readings were taken three times and average pH value was taken as final.

3) Viscosity-

The viscosity of F1 F2 F3 batches was evaluated by using Brookfield viscometer at spindle no 4 at 10 rpm

4) Spreadability-

The spreadability is evaluated for 3 batches by measuring its spreading diameter between two horizontal plates for one minute.

5) Drug content-

For drug content evaluation, 1gm of emulgel is added to the 100ml volumetric flask containing 70 ml of 50% ethanol. Then volume was made up to 100 ml by using 50% ethanol followed by 2 hrs. Shaking by mechanical shaker. Then it was filtered through filter paper then 1 ml filtrate was pipette out. Blank solution is prepared. The extract is estimated spectrophotometrically by UV spectrophotometer.

6) In-Vitro diffusion study –

The diffusion study of prepared emulgel was carried out by using Franz Diffusion cell by using the egg membrane. Emulgel sample 0.5 gram was taken on egg membrane and carried out-diffusion studies at 37 degrees Celsius using phosphate buffer as pH 7.4 as diffusion Medium. One ml sample was withdrawn periodically for 15min, 30min, 45min, 1hr, 2hr, 3hr and 4 hr., 5hr and 6hr then each one ml sample was replaced with an equal volume of buffer and checked the absorbance by using a UV spectrometer at 266 nm wavelength.

(Egg membrane removal method for study – – Egg was deep in Conc. HCl for 30 minutes. Due to this, the outer membrane of an egg will be dissolve. The membrane will look transparent. After that, the inner content of the egg will be removed by pricking at one point on the surface of an egg. Then the membrane is washed with water and used for diffusion study)

7) Microbial assay –

The cup plate method is used to perform a microbial assay. In this microbial assay FTM medium is used. The activity of herbal emulgel is evaluated against *Propionibacterium acnes.* The frizzed culture of *P. Acnes* has been incubated anaerobically which was purchased from MTCC Chandigarh. The bacteria were checked under an electron microscope for the confirmation. Emulsion has evaluated by presence of zone of inhibition.

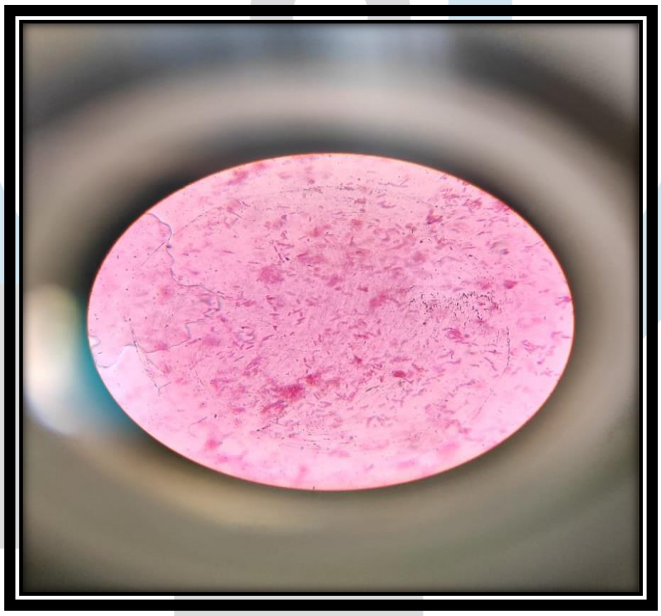

FIG 8: Microscopic view of *Propionibacterium acnes*

RESULT AND DISCUSSION

1) Physical parameters-

The physical parameter of prepared emulgel was checked visually for 3 batches. The results are shown in table no 8

Table No 10: results for color, phase separation, grittiness and homogeneity

SR NO	FORMULATION NO	COLOUR	PHASE SEPARATION	GRITINESS	HOMOGENECITY
1	1	Light Yellow	Phase Separation seen	No	Not Homogeneous
2	2	Light Yellow	None	No	Homogeneous
3	3	Light Yellow	None	NO	Homogeneous

2) pH-

The pH of the formulation was found to be in the range of 6.8 to 7.0.This range is lies within the normal pH range for skin and would not produce any irritancy to the skin when applied The pH values of 3 batches are shown in Table no 9

3) Viscosity-

Viscosity of emulgel shows the consistency. The decreased in consistency of emulgel shows increased in drug release. The viscosity of 3 batches is shown in table no 9

4) Spreadability-

The spreadability of the emulgel formulation was determined after 24 hrs. after permeation. It is calculated by measuring the spreading diameter of 1 gm. of emulgel between 2 horizontal plates (20*20 cm) after one minute. The Voltaren emulgel used as reference standard The first two batch shows better spreadability than last one The values of spreadability is shown in table no 9

5) Drug Content-

The drug content of drug was found to be 88.70 to 94.60% which is shown in Table no 9

Table No 11: Results for pH, Viscosity, Spreadability and drug content

SR NO	FORMULATION BATCH	pH	VISCOSITY (In CPS)	SPREADABILITY (In mm)	DRUG CONTENT (%)
1	F1	6.8	1340	40.30	90.30%
2	F2	7.0	1310	55.50	94.60%
3	F3	6.9	1322	35.60	88.70%

6) In vitro diffusion study-

In vitro diffusion study was done for 3 batches i.e. F1, F2 & F3 for 6 hrs. The percentage of cumulative drug released at different time interval for 3 batches is shown in table no 10 .In vitro diffusion study carried out in diffusion cell for 6 hrs.

Table No 12: **In vitro Diffusion Study % CDR**

Time(min)	F1 % CDR	F2 % CDR	F3 % CDR
0	0	0	0
15	6.6667	9.259	6.667
30	16.667	18.811	13.934
45	24.8	28.811	22.422
60	33.4	39.111	31.552
120	42.067	49.855	41.812
180	50.867	60.966	53.333
240	59.867	73.556	66.066
300	69.533	86.512	80
360	79.533	99.855	95.155

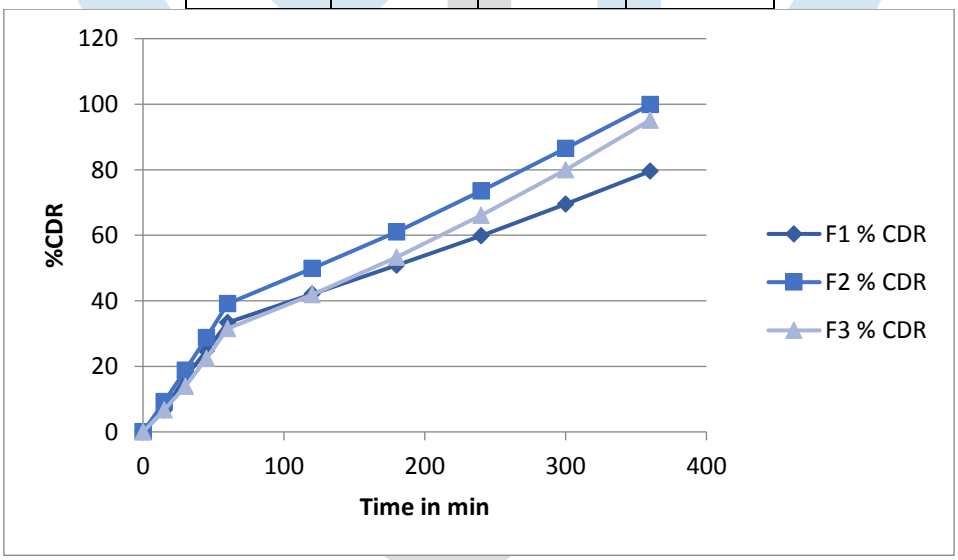

FIG 10: Drug release profile for batches F1 F2 & F3

7) Microbial Assay-

The zone of inhibition was seen against the Propionibacterium Acnes.

Discussion:

On the basis of results of evaluation parameters of the Batch No F2. It was found to be optimized because it has shown the no phase separation, grittiness, no colour change and homogeneous. The result of F2 is shown in following table no 11

Table No- 13: Results of Batch F2

Evaluation Parameter	**Result**
pH	7.9
Viscosity	1310
Spreadability	55.50
Drug Content	94.60%

% CDR (at 360 min)	99.855%
Microbial Assay	Zone of inhibition has observed which concludes that, formulation is having anti-acne activity.

Conclusion

In upcoming years, topical drug delivery will be used extensively to impart better patient compliance. Since emulgel helps to enhance spreadability, adhesion, viscosity. This novel drug delivery system will become popular, because, it will become a ray of hope for loading hydrophobic drugs in water-soluble gel bases for the long-term stability. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than synthetic ones. So, the herbal anti-acne formulation is safe, effective and improves patient compliance. The present study has undertaken to formulate and evaluate the herbal emulgel formulation prepared from ethanolic extract of stems of B. aristata. and ethanolic extract of Propolis which has also used as an antioxidant. Thus, the present research work shows that, herbal emulgel of B. aristata is having effective anti-acne activity which has been proved on the basis of different evaluation parameters for emulgel. The prepared emulgel comply with the pharmacopoeial standards, followed by a stability study conducted for optimized batch stored at 40°C/75% RH for one month. Emulgel was evaluated after one month for different parameters such as appearance, feel on application, pH, and viscosity. It has concluded that the above-selected formulation was stable.

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