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HERBALLY MEDICATED LIPOSOMAL GEL FOR ACNE VULGARIS

Vandana Ramesh* and K.V. Arun Kumar

Department of Pharmaceutics, Rajiv Gandhi Institute of Pharmacy, Trikaripur (P.O) Kasargod (Dist), Kerala -671310.

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***Corresponding Author**
Vandana Ramesh
Department of Pharmaceutics, Rajiv Gandhi Institute of Pharmacy, Trikaripur (P.O) Kasargod (Dist), Kerala - 671310.

ABSTRACT

The development of a novel drug delivery system for herbal drugs has a greater impact on today's world population as it safe, effective, convenient and economically affordable. Liposomal formulations have successfully used in the treatment of various dermatological diseases. Both synthetic and herbal drugs are incorporated into liposomes to improve its efficacy. *Symplocos racemosa*, *Rubia cordifolia* and *Azadirachta indica* possess good anti-acne activity and their synergistic action when incorporated into liposomes have been studied. Liposomes were prepared by Lipid Film Hydration method and the drug extracts were incorporated into it. Liposomes were assessed for particle size determination and the mean particle size was found to be in between 2.77µm to 5.70 µm. The pH of the formulated gel was compatible with the pH of the skin. The viscosity, spreadability and swelling index studies also showed better results. This drug loaded liposomes are then added into carbopol solution and formulated into a liposomal gel. The formulated liposomes were characterized by SEM. The gel formulation F4 containing CH:SL-1:2 was found to be optimized. All the formulated gel were subjected for anti-bacterial studies and F4 showed comparatively better results. This formulated gel F4 was then compared with the standard Clindamycin gel. Stability studies indicated that the formulation was stable over a period of 3 months at the 25±2^{o}C and 2-8 ±3^{o}C. It was observed that the drug loaded liposome was more efficient against *Propionibacterium acnes.*

KEYWORDS: Herbal drug, Liposomes, Acne vulgaris, Drug extracts, *Propionibacterium acnes.*

INTRODUCTION

Herbal drugs frame a major role in all the traditional systems of medicine and is regarded as a triumph of popular therapeutic diversity. Plants are being used in medicine from primeval period because they have cooked up the immediate personal needs, attainable and inexpensive. The use of plants for medicinal purposes around the world still tremendously exceeds the use of modern synthetic drugs. The medicinal plants have not only been known to be the backbone of all systems of medicine but have been finding extensive place in household remedies, nutraceuticals, natural drug molecules, natural dyes, mucilages, gums, phytochemicals, insecticides, pesticides and cosmetics.The quality of the raw materials used in herbal drugs affirms special significance particularly in setting experimental studies, clinical trials and their respective therapeutic value.

The cosmetics, according to the Drugs and Cosmetics Act is defined as articles intended to be rubbed, poured, sprinkled or sprayed on, introduced into or otherwise applied to the human body or any part there of for cleansing, beautifying, promoting attractiveness or altering the appearance.[1] Plants are extensively used for development of new drug products for cosmaceuticals and pharmaceutical application.[2] In herbal cosmetics herbs are used both in crude or extract form.[3] Herbs do not shows a quick heal instead they offer a way to put the body in proper tune with nature.[4] An enormous number of cosmetic and toiletry formulations have been designed and developed based upon Indian Herbs recently. There is an elevated demand for herbal medicine due to their skin friendliness and lack of side effects. The better thing in opting herbal cosmetics is that it is purely made by the herbs and shrubs and thus it is side effects free.

Acne vulgaris is a common skin condition, causing changes in pilosebaceous units (PSU) and skin structure consisting of a hair follicle and its associated sebaceous gland, via androgen stimulation. It is characterized by non-inflammatory follicular papules or comedones and by inflammatory papules, pustules and nodules in its more severe forms. Acne vulgaris is one of the most acquainted skin disorder affecting more than 85% population of the world, specifically teenagers and adolescents. Acne is a common disease that in cases of extreme disfiguration can have severe consequences for the personality development of young people and is associated with a relatively high prevalence of depression and suicide.[5] Spontaneous regression is common, but acne can extend into the fourth and fifth decades of life. Acne is an inflammatory disease of sebaceous follicles of skin marked by comedones, papules,

pustules, nodules and presence of bacterias such as *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Malassezia furfur* in follicular canal.[6]

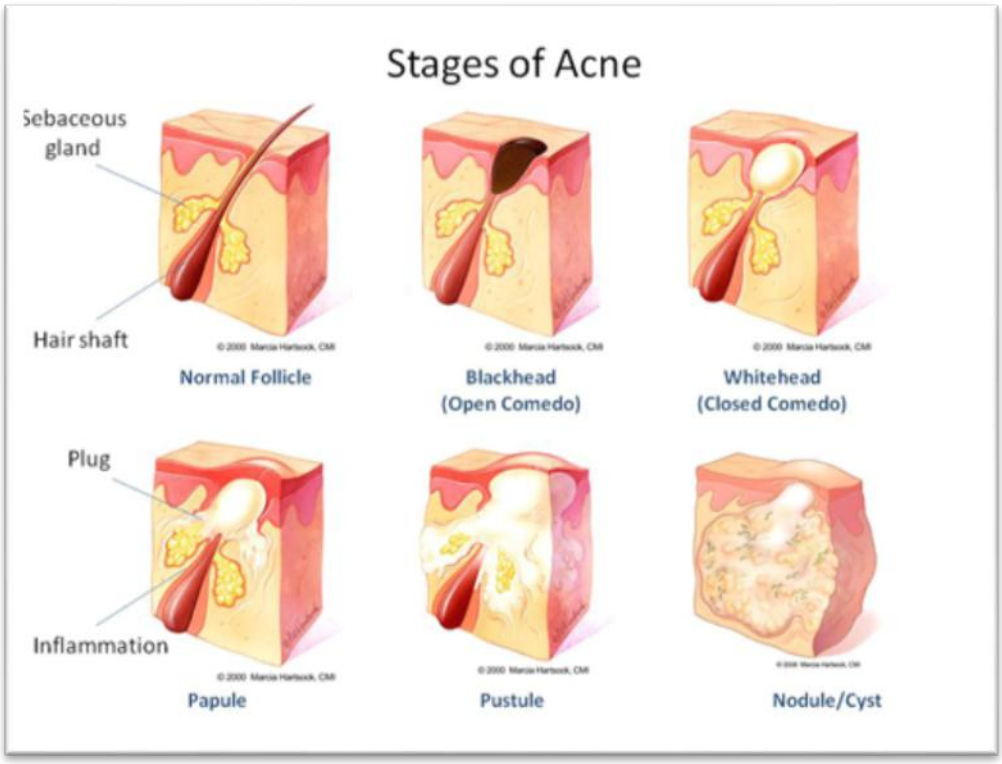

Figure 1: Stages of acne vulgaris.

P. acnes bacteria live deep within follicles and pores, away from the surface of the skin. In these follicles, *P.acnes* bacteria use sebum, cellular debris and metabolic byproducts from the surrounding skin tissue as their primary sources of energy and nutrients. Elevated production of sebum by hyperactive sebaceous glands (sebaceoushyperplasia) or blockage of the follicle can cause *P. acnes* bacteria to grow and multiply. *P.acnes* bacteria secrete many proteins, including several digestive enzymes.These enzymes are involved in the digestion of sebum and the acquisition of other nutrients. They can also destabilize the layers of cells that form the walls of the follicle. The cellular damage, metabolic byproducts and bacterial debris produced by the rapid growth of *P. acnes* in follicles can trigger inflammation. This inflammation can lead to the symptoms associated with some common skin disorders, such as folliculitis and acnevulgaris. The damage caused by *P. acnes* and the associated inflammation make the affected tissue more susceptible to colonization by opportunistic bacteria, such as *S.aureus.*[7] Preliminary research shows healthy pores are only colonized by *P.acnes*, while unhealthy ones universally include the non-pore resident *Staphylococcus epidermidis*,

amongst other bacterial contaminants. Thus *P.acnes* and *S.epidermidis* are the target sites for anti-acne drugs.

Development of an existing drug molecule from a conventional form to a novel delivery system can significantly improve its performance in terms of patient compliance, safety and efficacy. NDDS refers to the approaches, formulations, technologies and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. NDDS is a combination of advance technique and new dosage form which are far better than conventional dosage forms.

Drug delivery systems often depends up on a carrier to negotiate these goals. Drugs are effective once they reach the diseased area but require sustenance in their delivery. Studies on drug carriers mainly point out on developing a vehicle for current drugs that will allow the drug to be targeted and then released from the carrier at the diseased site. An ideal carrier would be able to detain a useful concentration of drug, to protect the drug from degradation, and to escape early removal from the body. Some carriers are individually attached to drug molecules while other systems enclose the drug in a capsule.[8] Liposomes are the most attractive candidates for drug delivery carriers because of their biocompatibility. The choice of particle to be researched depends on which property is more important for the system being considered. Other variables, such as ease of production and modification of surface properties, must also be considered.[9,10]

Liposomes were artificially prepared vesicles of spherical shape that can be created from cholesterol and non-toxic phospholipids. Both hydrophilic or hydrophobic drugs can be filled into the liposomes. Drug molecules can either be encapsulated in the aqueous space or setdown into the lipid bilayer. The physicochemical characteristics and the composition of the lipids will determine the exact location of a drug in the liposome. Liposome are also known as concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a lipid bilayer membrane usually composed of natural or synthetic phospholipids. Liposomes consist of relatively biocompatible and biodegradable nontoxic material, and they contain an aqueous volume entrapped by one or more lipid bilayer membrane.[11,12]

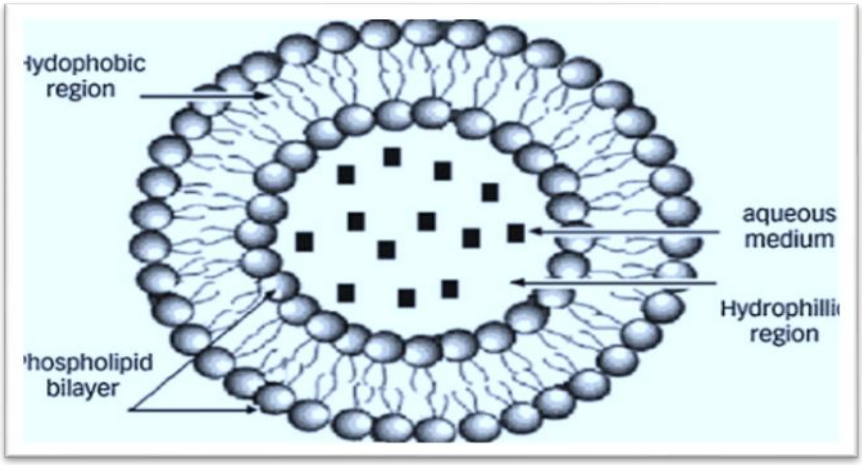

Figure 2: Cross section of liposomes.

Presently NDDS have been widely utilized only for allopathic drug, but they have their own limitations hence, turning to safe, effective and time tested Ayurvedic herbal drug formulation would be a preferable option.The use of herbal medicines has been increased globally due to their incredible therapeutic effects and fewer side effects as compared to modern medicines. However, delivery of herbal drugs also requires certain modifications with the intention to achieve sustained release to increase patient compliance etc. Formerly, herbal drugs fails to captivate scientist towards the development of NDDS due to processing, standardizing, extracting and identification difficulties. But recently with the advancement in the technology NDDS opens the door towards the development of herbal drug delivery systems. Novel Drug Delivery Technology have attained the importance to achieve modified delivery of herbal drugs therapy increasing the therapeutic value and reducing the toxicity. For the last one decade many novel carriers such as liposome, nanoparticles, phytosomes and implants have been reported for successful modified delivery of various herbal drugs.

Herbal drug or plant actives possess a lot of therapeutic potential that should be canvassed via application of NDDT. Large molecular size, lipid solubility, degradation in acidic stomach, are certain drawbacks which limit the therapeutic activity of these extracts invivo though these possess excellent bioactivity invitro. Application of NDDS led to enhanced bioavailability of plant actives by increasing the permeability and solubility as well as minimizing the side effects. A number of plant constituents like alkaloids, glycosides, flavanoids, tannins etc showed enhanced therapeutic effect at similar or less dose when incorporated into NDD vehicles as compared to conventional plant extracts. Hence there is a terrific potential in development of NDDS for priceless herbal drugs as it provides efficient

and economical drug delivery and the trends of incorporating NDDS for herbal drugs have also been adopted at industrial scale.

MATERIALS AND METHODS

Collection of plants

The bark of *Symplocos racemosa* (Roxb), roots of *Rubia cordifolia* (Linn), fresh leaves of *Azadirachta indica* A.Juss were collected from Kannur district, Kerala (India) in the month of October 2016. The plant materials were identified and authenticated in the Department of Horticulture, Padannakad, Kasargod, Kerala.

Preparation and of plant extracts[13]

The collected plant materials were carefully washed under running tap water followed by sterilized distilled water and were air dried at room temperature for 30-45 days. These dried plant materials were then homogenized to a fine coarse powder using an electronic blender and then stored in air tight containers until further use. Various organic solvents viz. ethanol, chloroform, petroleum ether and water were used for extraction. 10gm of homogenized coarse powders of bark of *S.racemosa*, roots of *R.cordifolia* and dried leaves of *A.indica* were soaked in different conical flasks containing 100ml of ethanol, chloroform, petroleum ether and water. All the three were allowed to stand for 30 min on a water bath with occasional shaking; finally each sample extract (ethanol, chloroform, petroleum ether and water) was filtered through sterilized Whatman No:1 filter paper and concentrated to dryness. Thus the obtained dried extracts were stored at 4^{o}C in labelled sterile bottles until further use. Phytochemical screening of the prepared drug extracts were performed and the results are shown below.

Preliminary Phytochemical Screening[13,14,15]

A) Detection of carbohydrates: Small quantities of extracts were dissolved in 5ml of distilled water and filtered separated. The filtrates were used to test the presence of carbohydrates.

- **Molish's test**: Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

- **Benedict's test**: Filtrates were treated with benedict's reagent and heated on water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.
- **Fehling's test**: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

B) Detection of alkaloids: Small portions of extract were separately stirred with dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloidal reagents.

- **Mayer's test**: Filtrates were treated with Mayer's reagent (potassium mercuric iodine). Formation of an yellow cream precipitate indicates the presence of alkaloids.
- **Wagner's test**: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide) and observed. Formation of brown / reddish brown precipitate indicates in presence of alkaloids.
- **Dragendroff's test**: Filtrates were treated with Dragendroff's reagent (Solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.
- **Hager's test**: Filtrates were treated with Hager's reagent (Saturated picric acid solution). Formation of yellow coloured precipitate indicates the presence of alkaloids.

C) Determination of Anthraquinones

- **Borntrager's test:** 5ml of each plant extract is to be shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. When the mixture is to be shaken to appear a pink, red or violet color in the ammonical (lower) phase indicates the presence of free hydroxyl-anthraquinones.
- **Modified Borntrager's test**: 2ml of test sample and 4ml of alcoholic KOH, dilute with 4ml of water and filter, then acidify with HCl. Next to cool and shake well with 5ml of ether. To separate the ether into test tube and shake with 2ml of dilute solution of NH_4OH. Development of rose red to intense red color indicates the presence of anthraquinones.

D) Determination of Glycosides

- **Legal's test:** The test samples were treated with pyridine and sodium nitroprusside solution to developed blood red color.

- **Kellar Killiani test:** 1ml of concentrated H2SO4 was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of FeCl3 were added, formation of a blue color indicates presence of glycosides.
- **Concentrated Sulphuric acid test**: When few ml of Con.H2SO4 was added to test sample gives reddish color indicates presence of glycosides.
- **Molisch test:** When naphthol and concentrated H2SO4 were added to test samples reddish violet ring at the junction of two layers was resulted, responding positive for the presence of glycosides.

E) Detection of saponins

- **Forth test**: Dilute the extracts with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

F) Detection of steroids

- **Liberman Buchard's test:** The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown ring at the junction indicates the presence of steroids.
- **Salkowski's test**: The extracts were treated with chloroform and filtered separately. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to stand. The formation of a yellow coloured lower layer indicates the presence of free steroids.

G) Determination of Tannins

- **Gelatin test:** Gelatin and water were added to test samples in a test tube to formation of white precipitate was resulted, indicating presence of tannins.

H) Determination of Phenols

- **Ellagic acid test**: When 5% glacial acetic acid and 5% sodium nitrite were added to extracts a muddy Niger brown color appears, which is a positive result for phenols.
- **Ferric chloride test:** Treat the extracts with few drops of neutral ferric chloride solution. The formation of Bluish black colour indicates the phenolic nucleus.

I) Determination of Flavonoids

- **Alkaline reagent test**: Few ml of test sample was taken and NaOH solution was added to form intense yellow color, which turns into color less on addition of few drops of dilute acid indicating the presence of flavonoids.
- **Lead acetate test**: When aqueous basic lead acetate was added to test sample produces reddish brown precipitate, indicating the presence of flavonoids.

J) Detection of proteins and aminoacids.

- **Biuret test**: The extracts were treated with 1 ml of 10% sodium hydroxide solutions and heated. Add a drop of 0.7% copper sulphate solution to the above mixtures. The formation of purplish violet colour indicates the presence of proteins.
- **Millon's test**: The extracts were treated with 2 ml of million's reagent. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins.
- **Ninhydrin test**: To the extracts added 0.25% ninhydrin reagent and boiled for few minutes. Formation of blue colour indicates presence of aminoacids.

Extraction of plant materials[16]

The extraction of dried barks of *Symplocos racemosa* Roxb, roots of *Rubia cordifolia* Linn and dried leaves of *Azadirachta indica* A.Juss were carried out by sequential hot soxhlet extraction process by using ethanol as solvent. The extracts obtained from all the three plant materials were collected and concentrated. It was then weighed and the dried extracts were kept in a desiccator which was previously filled with fused calcium chloride until it was used for the formulation of liposomal gel.

Preformulation study[17]

- **Drug- Excipient Compatibility Studies by FT-IR**

The interaction studies were carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of gel formulation. Fourier-transform infrared spectra were obtained by using JASCO FT-IR 4700 L spectrometer.

Preparation of liposomes and liposomal gel[5,18,19,20]

Liposomes were prepared using lipid film hydration technique with various concentrations of soyalecithin and cholesterol. Soyalecithin, cholesterol and drug extracts were accurately weighed then dissolved in 15 mL mixture of chloroform: methanol (2:1 v/v ratio). Above mixture was sonicated for 5 min. Then it was vortexed in a round bottom flask at a

temperature of 58-64°C to remove the solvent for about 30 min. The thin lipid layer formed inside the flask was then hydrated with 10 mL of 7.4 Phosphate buffer at 60°C for 1hr. Dispersion was left undisturbed at room temperature for 2-3 hours to allow complete swelling of the lipid film and hence to obtain vesicular suspension.

Table 1: Composition of developed liposomes.

Formulation code	Weight taken in mg					Cholesterol: Soyalecithin
	S.racemosa	*R.cordifolia*	*A.indica*	Cholesterol	Soyalecithin	
F1	300	50	50	100	100	1:1
F2	300	50	50	200	100	2:1
F3	300	50	50	200	200	2:2
F4	300	50	50	100	200	1:2
F5	50	300	50	100	100	1:1
F6	50	300	50	200	100	2:1
F7	50	300	50	200	200	2:2
F8	50	300	50	100	200	1:2

The optimized liposomal formulation was used for the preparation of 1% carbopol gel. The required amount of carbopol 934 (1% W/W) was weighed and slowly sprinkled into a 500ml beaker containing distilled water with continuous stirring using mechanical stirrer (at the minimum speed to avoid entrapment of air) to get a transparent dispersion. After complete homogenization of the carbopol polymer with distilled water prepared liposomal dispersion is added into the above mixture slowly with continuous stirring followed by addition of 10 % propylene glycol. Required amount of preservatives were taken in a beaker and is dissolved by heating it over a water bath and is then added to the above mixture. The above dispersion is then neutralized using triethanolamine with continuous stirring for adjusting the skin pH (6.8-7) and to obtain a gel at required consistency.

Evaluation[49,53,57,58,75]

- **Vesicle size of liposome**

Liposomal suspension was exposed to ultrasonic irradiation with duration of 30 min in continuous sonication bath. The sample was left to cool down and placed in the fridge at 4^{o}C for 1 day prior to the test. The liposomal suspension was then tested for particle size analysis by microscopy. Sample of liposomal suspension was evaluated for particle size after suitable dilution. Optical microscopy was used with oil immersion lens. Diameters of 50 liposomes were measured and mean geometric diameter and standard deviation were calculated.

- **Vesicle shape of liposomes**

The shape and morphological characters were obtained from SEM photographs of the optimized liposomes. The SEM analysis of prepared drug loaded liposomes was subjected for morphological studies. The formulation were placed into circular aluminium stubs using double adhesive carbon tape and coated with gold in Hitachi ion sputter E-1010 vaccum evaporator. Then it was observed in Hitachi SU6600 FE SEM (Field Emission Scanning Electron Microscope) having acceleration voltage of 10.0Kv and magnification of 100k.

Evaluation of liposomal gel[16,18,19,20,21]

A. Physiochemical Evaluation

- **Physical examination:** The herbal liposomal gel was prepared by the procedure mentioned and evaluated for colour,odour and transparency.
- **pH:**The pH of various gel formulations were determined by using digital pH meter. 2.5 g of gel was accurately weighed and dispersed in 25ml distilled water and stored for 2 hours. The measurement of pH of each formulation was done in triplicate and average values are noted.
- **Spreadability:** An important criteria for semisolids is that it posses good spreadability. Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends on its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spreadability. 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 and was sandwiched between the two slides across the length of 5cm 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 slide 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 a simple pulley and a pan. A 30g 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.

Spreadability = MxL/T

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

L= length of glass slide (5cm)

T=time taken in seconds.

- **Viscosity:** Viscosity of the gel was determined using Brookfield viscometer (DV-1 programmable rheometer) at 6rpm. Gels were tested for their rheological characteristics at 25^{o}C. 200 g of the gel was taken in a beaker and spindle was dipped in it for about 5 minutes and then the reading was taken.

- **Swelling Index:** Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared topical gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1N NaoH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows.

Swelling Index (SW) %=$\frac{Wt-W0}{W0} * 100.$

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

- **Homogeneity:** Developed liposomal gel was tested for homogeneity by visual inspection after the gel has been set in the container. This was tested for their appearance and presence of any aggregates.

- **Extrudability studies:** The gel formulation were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of tubes were recorded and the tubes were placed between two glass slides and were clamped. 500gm were placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel was calculated as.

1) When it is greater than 90% then the extrudability is excellent.
2) When it is greater than 80% then the extrudability is good.
3) When it is 70% then the extrudability is fair.

- **Washability:** The product was applied on hand and was observed under running water.

B) Invitro Studies

- **Anti microbial study:** ***Agar plate well diffusion method***

Anti-acne susceptibility was done by agar plate well diffusion method. This method involves sterilization of petri plates, seeding of medium, inoculation and incubation. The plates were sterilized by dry heat in an oven at 160^{o}C for one hour. Broth for each bacterial agar was prepared and sterilized by autoclaving. Molten agar (25ml) was poured in each petri plate. The test tubes were cooled upto 50^{o}C, and then 20ml of molten agar from each test tube was added in sterile petriplates aseptically and kept to solidify. Standard cultures of microbes were poured on top of the agar n the plates. After solidifying, wells of 5mm were bored aseptically using a sterile cork borer. The agar plugs were taken out carefully so as not to disturb the surrounding medium. The holes were filled completely with desired formulation. The plates were kept in an incubator at 37^{o}C for specified time. After this petri plates were observed for the antibacterial activity by measuring the zone of inhibition. Clindamycin gel was used as a standard.

- **Stability Studies of optimized formulation**

The optimized formulation was subjected for stability studies. Optimized herbal liposomal gel was sealed in amber coloured bottles with cap covered by aluminium foil and these packed formulations was stored in different temperature viz a) room temperature (R.T) b) 2^{o}C-8^{o}C±3^{o}C and according to ICH guidelines, maintained at 25^{o}C±2^{o}Cfor 3 months. The formulation was evaluated before and after periodic interval for change in appearance, pH, viscosity and anti-microbial studies.

RESULTS AND DISCUSSIONS

The collected plant materials was extracted using ethanol as solvent and this ethanolic extracts were subjected for phytochemical studies to determine the biologically active constituents.

Preliminary Phytochemical Screening

Table 2: Phytochemical test on various extracts of *S. racemosa* bark powder

Phytochemical Test	Ethanolic extract	Chloroform extract	Petroleum ether extract	Aqueous extract
Carbohydrates	+++	+	-	+
Alkaloids	++	-	-	-
Anthraquinones	++	-	-	+
Glycosides	+++	-	+	-
Saponins	++	-	+	-
Steroids	+++	++	+	+
Tannins	+	-	-	-
Phenols	++	-	-	-
Flavanoids	+	-	-	-
Proteins and amino acids	+	-	-	-

Table 3: Phytochemical test on various extracts of *R. cordifolia* root powder.

Phytochemical Test	Ethanolic extract	Chloroform extract	Petroleum ether extract	Aqueous extract
Carbohydrates	++	-	-	-
Alkaloids	-	-	-	-
Anthraquinones	+++	+++	++	++
Glycosides	++	+	+	++
Saponins	++	+	+	+
Steroids	+++	++	+	+
Tannins	-	-	-	-
Phenols	++	+	+	+
Flavanoids	++	++	++	+
Proteins and amino acids	-	-	-	-

Table 4: Phytochemical test on various extracts of *A. indica* leaf powder.

Phytochemical Test	Ethanolic extract	Chloroform extract	Petroleum ether extract	Aqueous extract
Carbohydrates	+++	-	-	+
Alkaloids	++	+	+	-
Anthraquinones	-	-	-	-
Glycosides	+++	-	-	-
Saponins	++	-	-	-
Steroids	++	-	-	-
Tannins	+	-	-	+
Phenols	+++	-	-	++
Flavanoids	++	+	-	+
Proteins and amino acids	+++	++	+	+++

(+++) highly present,(++) moderately present,(+) present,(-) absent

The phytochemical test on ethanol, chloroform, petroleum ether and aqueous extracts of *Symplocos racemosa* bark powder, *Rubia cordifolia* root powder and *Azadirachta indica* leaf powder showed the presence of various phytoconstituents and ethanolic extracts showed better results as compared to the other three solvents. The present investigation reveals that ethanol can be used as menstrum for further extraction.

Extraction of plant materials

The percentage yield of extracts obtained for *Symplocos racemosa, Rubia cordifolia* and *Azadirachta indica* in ethanol were found to be 27.04%w/w, 24.02%w/w, 22.94%w/w.

Preformulation study

Drug- Excipient Compatibility Studies by FT-IR

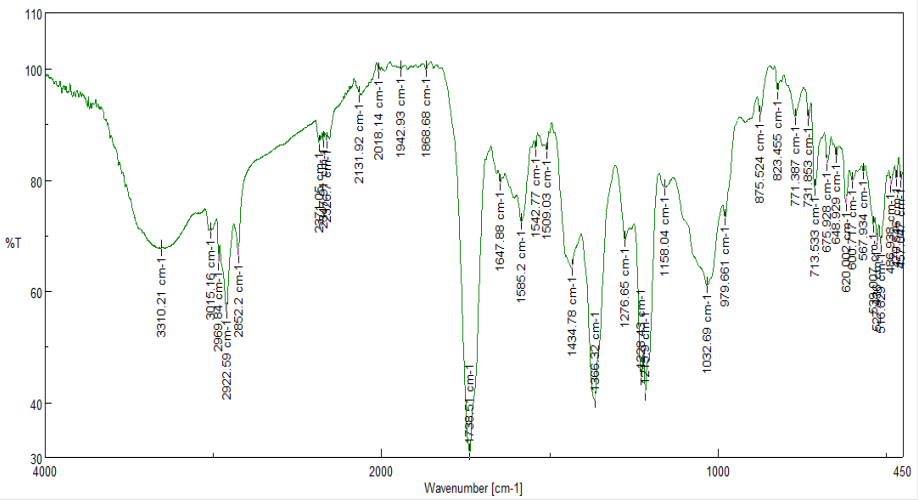

Figure 3 : FT-IR Spectrum of *Symplocos racemosa* Roxb.

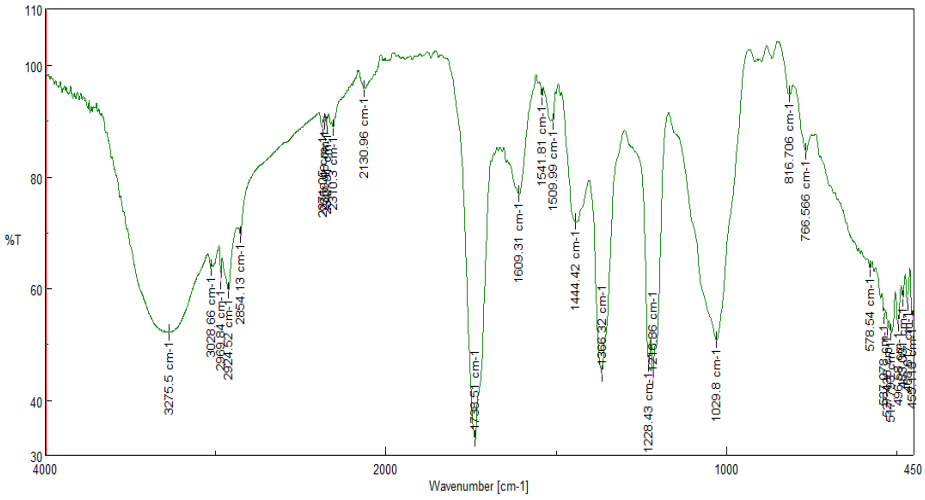

Figure 4: FT-IR Spectrum of *Rubia cordifolia* Linn.

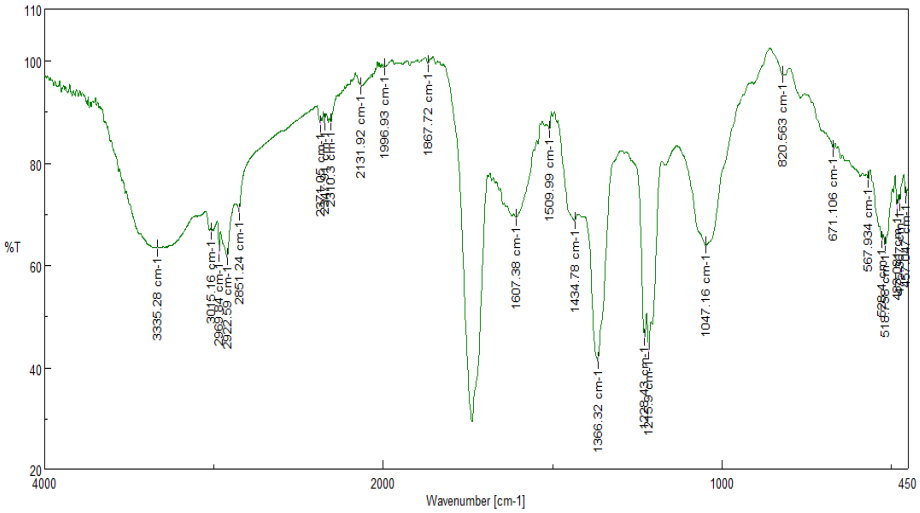

Figure 5: FT-IR Spectrum of *Azadirachta indica A. Juss.*

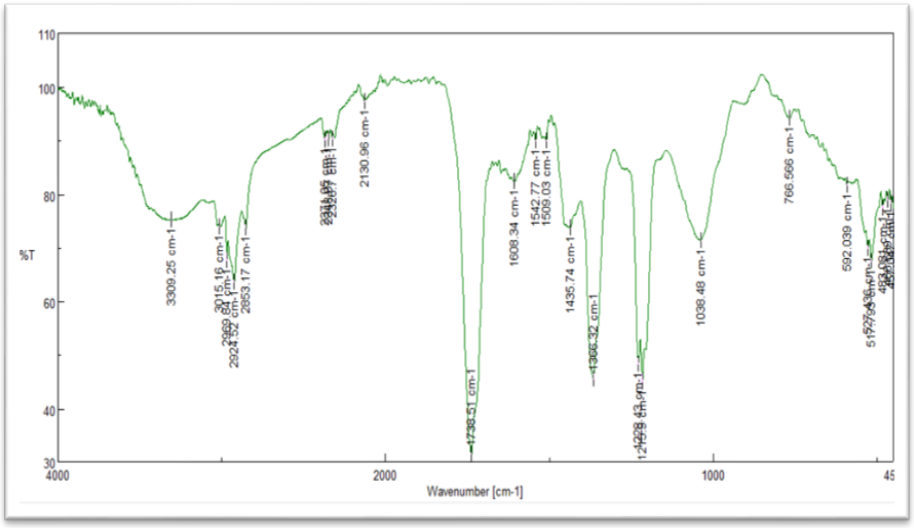

Figure 6: FT-IR Spectrum of drug samples + carbopol-934+ cholesterol.

All the above characteristic peaks of drug appear in the spectra of all other spectra of drug with polymer mixtures and formulations of liposomal gel at the same wave number, indicating no modification or interaction between the drug and the excipients. From this it can be concluded that the drug has maintained its identity without losing its characteristic properties. It will not show any adverse effect in action of the formulation and helps to study desired parameters in the present study.

EVALUATION

Vesicle size of liposomes

The liposomes were analyzed for their size distribution using a compound microscope at 100 X to observe for their spherical nature.

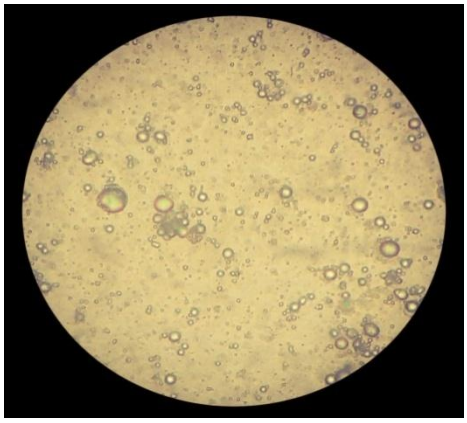

Figure 7: Optical microscopic images of drug loaded liposomes.

The diameter of the particle in formulation F4 was found to be 3.60µm ± 0.398.

Vesicle shape of liposomes

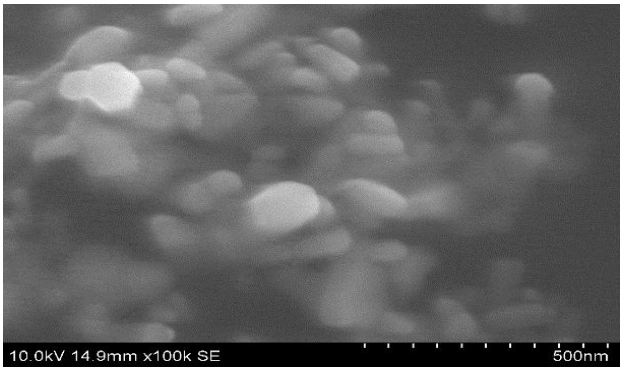

Figure 8: SEM photographs of formulated liposome.

Evaluation of liposomal gel

A. Physiochemical Evaluation

Table 5: Physical examination of liposomal gel.

Formulation code	Colour	Odour	Appearance
F1	Yellowish brown	Characteristic	Clear and translucent
F2	Yellowish brown	Characteristic	Clear and translucent
F3	Yellowish brown	Characteristic	Clear and translucent
F4	Yellowish brown	Characteristic	Clear and translucent
F5	Reddish brown	Characteristic	Clear and translucent
F6	Reddish brown	Characteristic	Clear and translucent
F7	Reddish brown	Characteristic	Clear and translucent
F8	Reddish brown	Characteristic	Clear and translucent

pH

The pH values of all formulated gel formulations ranges from 5.75 to 6.95 which lies in the normal pH range of skin.

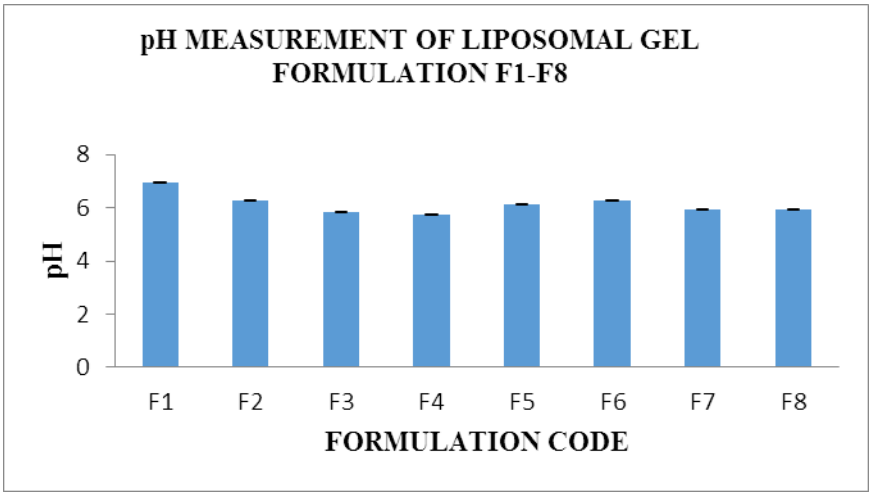

Figure 9: pH measurement of developed liposomal gel.

Spreadability and Viscosity

The values of spreadability indicate that the facewash gel is easily spreadable by small amount of shear. Shorter the time interval to cover a distance of 5 cm indicates better spreadability.

Spreadability and viscosity are interrelated. The formulation with higher spreadability will show minimum viscosity.

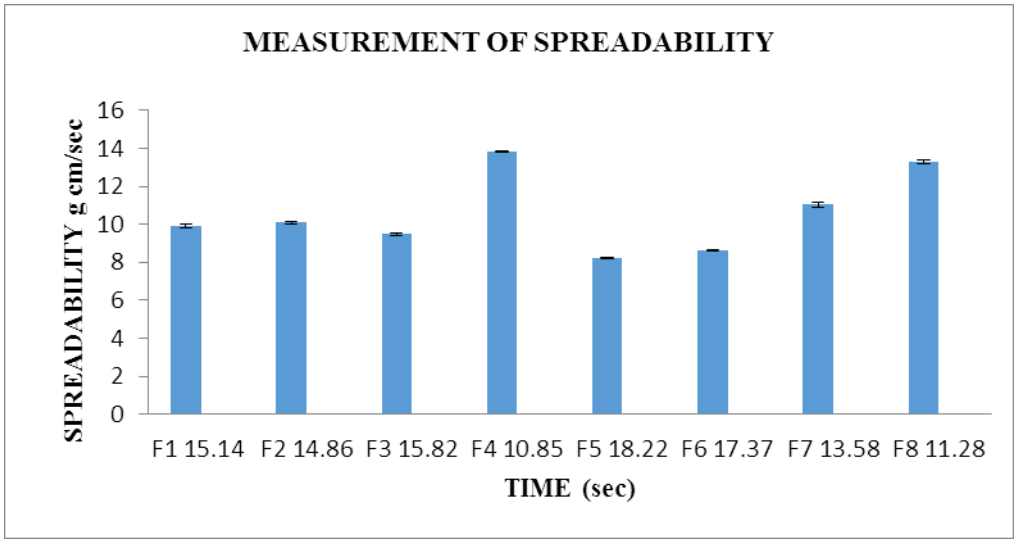

Figure 10: Measurement of spreadability of developed liposomal gel.

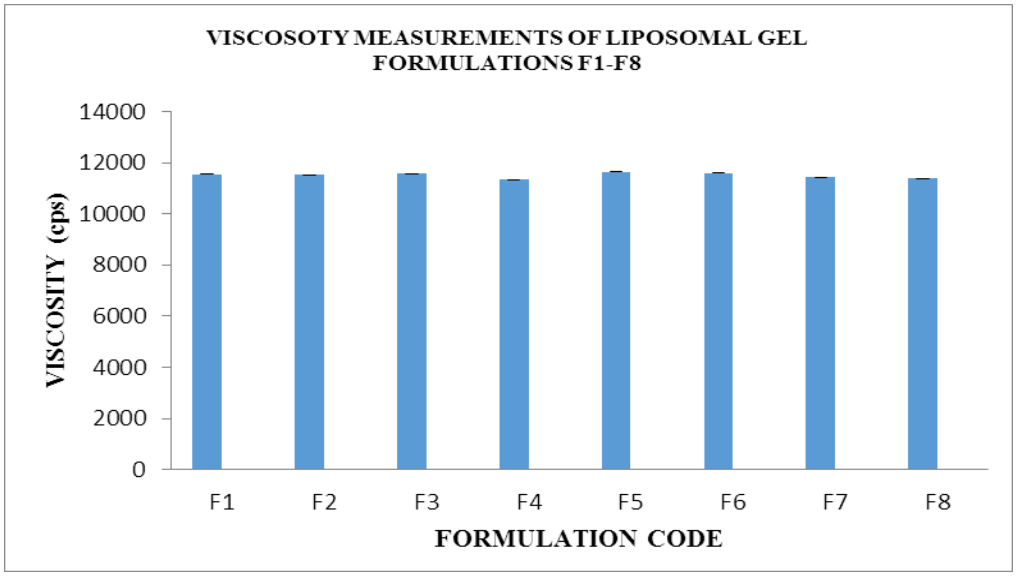

Figure 11: Measurement of viscosity of developed liposomal gel.

From formulation F1to F8, F4 showed a better spreadability value of 13.82±0.03 in a shorter time of 10.85 sec with a minimum viscosity of 11340.02 ± 0.23.

SWELLING INDEX

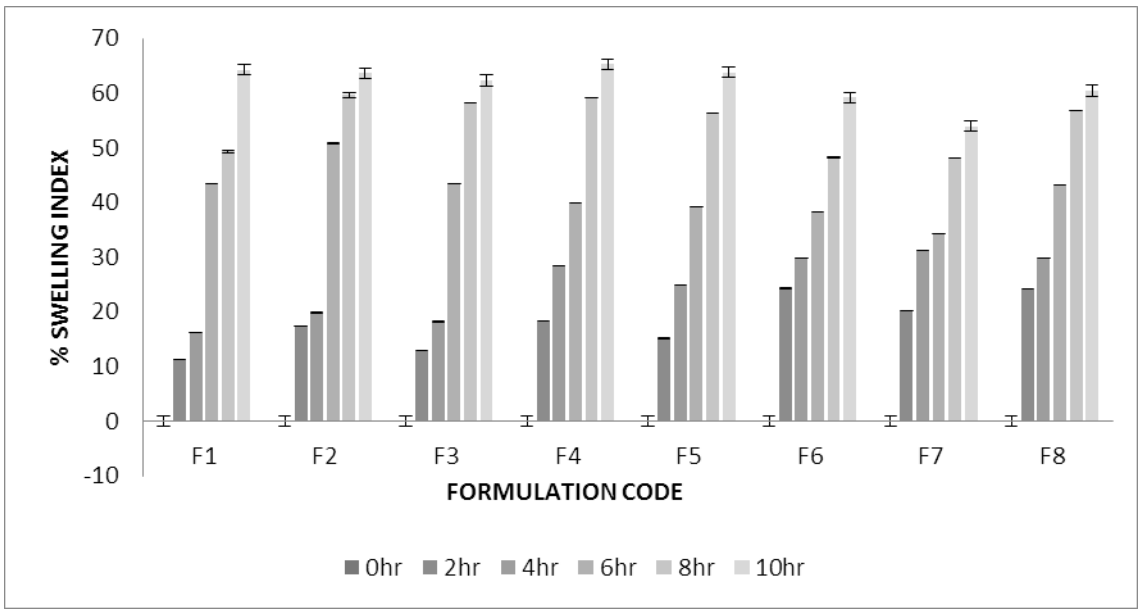

Figure 12: Swelling index study.

All the developed formulations shows good swelling index value of which better swelling index at a defined time intervalwas shown by formulation F4.

Table 6: Homogenity, washability and extrudability studies

Formulation code	Homogeneity	Washability	Extrudability
F1	Homogenous	Good	Excellent
F2	Homogenous	Good	Good
F3	Homogenous	Good	Fair
F4	Homogenous	Good	Excellent
F5	Homogenous	Good	Good
F6	Homogenous	Good	Good
F7	Homogenous	Good	Good
F8	Homogenous	Good	Excellent

B) Invitro Studies

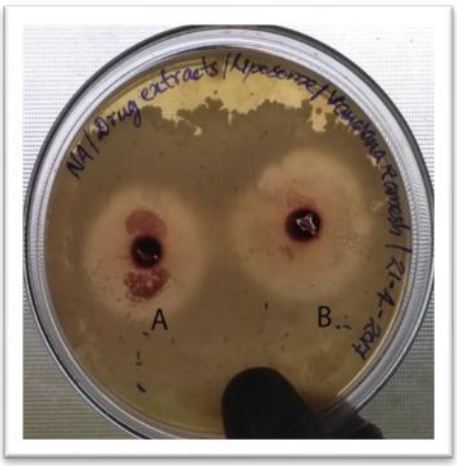

Figure 13: Comparison of Zone of inhibition of normal gel and liposomal gel.

In the above figure A- ZOI produced by normal gel.

B-ZOI produced by liposomal gel.

Table: Measurement of zone of inhibition.

Sl No	Test samples	Zone of inhibition (mm) for *P.acne*
1	*S.racemosa* extract(10mg/ml)	10.88 mm
2	*R.cordifolia* extract (10mg/ml)	7.7mm
3	*A.indica* extract (10mg/ml)	5.7mm
4	Extract combination (30mg/ml)	14.1mm
5	Liposomal dispersion	16.5mm
6	Liposomal gel F1	25.6mm
7	Liposomal gel F2	29.4mm
8	Liposomal gel F3	32.8mm
9	Liposomal gel F4	33.5mm
10	Liposomal gel F5	20.8mm
11	Liposomal gel F6	18.7mm
12	Liposomal gel F7	17.2mm

13	Liposomal gel F8	23.5mm
14	Normal gel	35.3mm
15	Clindamycin gel	38.2mm

The formulated liposomal gels from F1 – F8 was subjected for anti – bacterial studies andF4produces comparatively better ZOI. Formulation F4 was compared with normal gel containing drug extracts and the same was compared with standard Clindamycin gel. Hence considering the results of all other evaluation parameters F4 was selected as the best liposomal gel for anti-acne activity and was then set aside for stability studies for 3 months. Stability parameters like visual appearance, pH, viscosity, spreadabilty, anti-microbial studies of the formulation showed that there was no significant variation during the study period.

CONCLUSION

The research area for novel drug delivery system in case of herbal drugs is an innovative work that target mainly the phytoconstituents and plant extracts regarding from the usefulness of the plant product particularly that containing anthraquinone glycoside, alkaloids, tannins, flavanoids, steroids etc. The drug loaded liposomes methods can be easily upgraded for commercial scale and has offered an excellent opportunity and hope in raising the invivo bioavailability of herbal drugs. Liposome loading not only reduce the frequent application to beat non-compliance, but also facilitate to extend the therapeutic value by reducing toxicity, enhancing the bioavailability and controlled release. As far as the benefits of liposome technology are concerned it is a great future for use in formulation technology and applications of both hydrophilic and lipophilic plant compound and extracts.

Further studies on invitro models are required to evaluate the potentials of herbally medicated liposomal gel formulation and then it can be useful for the clinical application

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