IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Development of Annona squamosa Extract Based Herbal Emulgel for Treatment of Psoriasis

Tanuja Wankhede[1], Unnati Kothawade[2]

Assistant Professor[1] , UG Student[2]
Department of Pharmaceutical Quality Assurance,

Mahatma Gandhi Vidyamandir's Pharmacy College, Panchavati, Nashik, Maharashtra, India.

ABSTRACT

Emulgels have emerged as promising topical drug delivery system for the delivery of hydrophobic drugs via skin. The objective of study was to prepare the drug Annuna squamosa leaf extract as emulgel. When we are preparing emulsions individually shows stability problems during manufacturing and storage that effect on drug release pattern. In order to increase the stability we are incorporated as emulgel. Annuna squamosa leaf extract are used for the treatment of Psorasis disease. Psoriasis is a hyper augmentation; an autoimmune skin disorder that influences 13% of the world's population. Psoriasis is a common skin circumstance that can be painful and itchy; between 1.5% and 3% of people in the world have psoriasisThe present study was to formulate and evaluate the topical herbal emulgel. In this study we develop emulgel form of Annuna squamosa leaf extract using Carbapol 940, Xanthum gum, Propylene glycol, Methyl paraben, Propyl paraben, Glycerine, Triethanolamine (to adjust pH), and Distilled water and evaluated for physical properties includes color, structure, solubility, homogenicity, consistency, swelling index DSC, FTIR and PH and in vitro drug release patterns, ex vivo release and stability study etc. among all the formulations F3 formulation showed superior drug release patterns than the remaining types of formulations of emulgels.

KEYWORDS

Annona squamosa leaf extract, carbopol 940 and Xantham gum., Antimicrobial, Disc diffusion.

INTRODUCTION

Psoriasis is a hyper augmentation; an autoimmune skin disorder that influences 13% of the world's population. Psoriasis is a common skin circumstance that can be painful and itchy; between 1.5% and 3% of people in the world have psoriasis. The skin consists of millions of tiny skin cells. Normally, skin cells die and are replaced by new ones every 3 to 4 weeks. In psoriasis, your body begins producing new skin cells more quickly than normal leading to raised patches. This is related to your immune response, which is how your body fights disease and heals wounds. A reaction to psoriasis is triggered by your immune system even though there is no infection or wound to heal. The reasons why it does this are not completely understood but they are usually due to variations in your genes. Researchers around the world are pursuing new, effective & safer medicines from natural resources to treat psoriasis because synthetic drugs are associated with severe side effects. Psoriasis is a chronic condition, not contagious, but psoriasis can affect all areas of the skin, including the scalp, nails, and genital area. This condition can also affect folds in the skin, such as under your arms, the inside of elbows and knees, or under your breasts. These areas are called flexural areas. Psoriasis can range from being a very mild condition to being a very severe one. At the moment there is no cure for psoriasis, but it can be well managed with various treatments.[1]

Mild to Moderate Psoriasis

Many patients with psoriasis have mild to moderate disease, affecting less than 5 percent of the skin surface area and sparing the genitals,hands, feet, and face. These patients can often betreated successfully with topical therapies including corticosteroids, vitamin D, steroid creams, and tazarotene and calcineurin inhibitors. There are a few less common topical therapies, such as no medicated moisturizers, salicylic acid, cool tar, and anthralin. A vitamin D analogy is used as immunotherapy or in combination with phototherapy to treat psoriasis in patients with 5 to 20 percent body surface involvement. These agents have a slow onset of action but a longer disease-free interval than topical corticosteroids. Tazarotene is a teratogenic topical retinoid.[2] Tazarotene is as effective as topical corticosteroids in reducing psoriatic symptoms, but it is associated with a longer disease-free interval. In general, they improve symptoms with less skin atrophy than topical corticosteroids and are considered first-line treatments for facial and flexural psoriasis.[2,3]

Severe Psoriasis

Patients with more severe psoriasis Necessities more than 5 % of the body surface area Or contain the hands, feet, face, or genitals are Generally treated with phototherapy in consonance With systemic therapies.[3] Systemic therapies Involve methotrexate, cyclosporine, acitretin, and Biologic therapies.[3,4]

TYPES OF PSORIASIS

Psoriasis is considered to be an immune-Mediated infection with a genealogical premise Including an aggregate between hyperplastic Epidermal keratinocytes and a small number of Immune cells compose of T-cells, neutrophils Dendrites cells, and macrophages. Psoriasis does Not expand starting with one individual, then onto

The next by contact, however, can be transmitted Hereditarily.[5,6] From a clinical perspective, Psoriasis can be viewed as a broad range of Different skin signs. The great part of the abrasion Has normal characteristics involving erythema, Thickening. Although the size of abrasion can Change from a pinhead up to a distance of 22 cm, Fringes of abrasion are normally round, oval, or Polycyclic. Even though it can impact any region, Knees, elbows, lumbo-sacral district, scalp, and Genital zone are a long time of interval involved.[7] Psoriasis is examined based on clinical history (skin rash, changes to nails, and joint association) And is normally clear. Sometimes, patients have Atypical skin abrasion that should be separated from Tinea, mycosis fungoides, discoid lupus, seborrheic Dermatitis, or non-particular skin signs, for Example, insignificant scaling of the scalp,segregated flexural erythema, or genital abrasion. Perceptive evaluation of all body surfaces may Reveal undeclared, diagnostically helpful highlights, And a skin biopsy may periodically be shown. A Different class of psoriasis is as follows and

Plaque psoriasis (psoriasis Vulgaris):

It is also known as severe stationary Psoriasis and involves 85–90% of people with Psoriasis. It's pitched up as raised red-colored Patches with silvery-white scales. This abrasion can Be very itchy and painful, and in severe conditions, It may even crack and bleed.Inverse psoriasis (flexural psoriasis): It is characterized by bright red, shiny Abrasion which emerges in skin folds such as Armpits, groin area, and under the breast. The Condition deteriorates as a result of fraction and Sweat, and it is susceptible to fungal infection.

Nail psoriasis:

35–40% of people can get psoriasis. It is Distinguished by pinhead-sized depression in the Nail, whitening of the nail, blood clots in capillaries Under the nail, yellow-red discoloration called oil Spot or salmon spot, subungual hyperkeratosis, and Onycholysis.

Erythrodermic psoriasis:

It is a rare type of psoriasis that Affectsonly3% of people with psoriasis. It can come In two varieties: First, progressive chronic plaque Psoriasis which grows in scope and confluent, and Second, erythroderma which as a result, the body's Thermoregulatory capacity is impaired, resulting in Hypothermia widespread inflammation of the Body's surface severe itching, pain, and swelling. This type of psoriasis is potentially fatal.

Pustular psoriasis:

It is distinguished by raised bumps filled With noninfectious pus or pustules. These pustules Are commonly found on the hands and feet, but They can also be generalized with random Widespread patches on any part of the body.

Guttate psoriasis:

In Latin, guttate means "drops." Guttate Psoriasis is characterized by small, red, and scaly Tear-shaped drops with silvery scales which appear On arms, legs, and the middle of the body. It is Usually seen in persons younger

than 30 It mostly Occurs after an acute B hemolytic streptococcal Infection of the pharynx or tonsil. The abrasion can Vary from 10 to 100.

Psoriatic arthritis: It is a chronic inflammatory arthritis that Frequently occurs in association with nails and skin Psoriasis. It is characterized by painful Inflammation of the joints and the surrounding Connective tissues. It can occur in any joint but Mostly affects the joints of the toe and fingers Which lead to dactylitis or sausage-shaped swelling On the fingers and toe. It can also affect the hips, Knees, and sacral.

PATHOPHYSIOLOGY OF PSORIASIS

There are two theories fundamentals Concerning the manners physiology of psoriasis. The primary hypothesis clarifies the advancement Of psoriasis because of the growth that is and Proliferation of the skin cells which is because of Hyper proliferation of the epidermal cells and Keratinocytes. In the second theory, T-cell- the Mediated immune system is the primary driver of Irritation which encourages overabundance of cell Development. Excessive production of the skin cell Is a secondary reaction to the factor generated by The immune system. Langerhans cell in the dermis Goes as an example, an antigen-presenting cell that Relocates to the lymph node (site of T-cell). White Blood cell actuation is caused by the presence of Langerhans cell as an unrecognized antigen and Because of co stimulatory signals. A co stimulatory Signal is caused by lymphocytes' function-related Antigen -3 and intracellular adhesive particles. Cytokines are discharged by T-cells in the dermis And epidermis because of the release of (tumor Necrosis factor) protesting aggregation and epidermal hyper expansion. Immunosuppressant clear out psoriasis and proof of support for the Immune-mediated model of psoriasis Pathophysiology.

EMULGEL

Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders [e.g. acne, psoriasis] with the intent of containing the pharmacological or other effects of drug to the surface of the skin or within. Topical drug administration through various routes applied a wide spectrum of preparation for both cosmetic and dermatological, to their health and diseased skin. Topical preparations are applied to the surface of a part of the body and have effects only in a specific area of the body and are formulated in such a manner that the systemic absorption of the medicament is minimal. The most common examples of topical dosage forms are solutions, suspensions, emulsions, semisolids [e.g. powders and aerosols] among them ointments, creams, and lotions have numerous disadvantages.They are usually very sticky and cause uneasiness to the patient when applied moreover they also have less spreading coefficient and need to apply with rubbing. They also exhibit the problem of stability. Due to all these factors, a major group of semisolid preparation, transparent gel has expanded its use in both cosmetics and in pharmaceutical preparation. Inspite of many advantages of gels a major limitation is their inability an emulsion based limitation an emulsion based

approach is being used so that a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels. When gels and emulsions are used in combined form the dosage forms are referred as emulgels.

Mechanism of Skin Penetration

Skin penetration enhancers are the molecules which reversible to remove the barrier resistance of the stratum corneum. They allow drugs to penetrate more readily to the viable tissues and hence enter the systemic circulation. The intercellular routes accelerants may interact at the polar head groups of the lipids, within aqueous region between lipid head group and between the hydrophobic tails of the barrier. The common mechanism is to protect the body for unwanted particles from the environment. The main barrier of the skin is located in the outermost layer of skin that is epidermis. Since the lipid regions in the stratum corneum forms the only continuous structure, substance applied on to the skin always have to pass these region. The major obstacle for topical drug delivery is the low diffusion rate of drug across the stratum corneum. Several methods have been assessed to increase the permeation rate of drugs temporarily.

Emulgel

Emulgels are emulsions, either of the oil-in-water or water in oil type which are gelled by mixing with gelling agent. Emulsified gel is stable one and superior vehicle for hydrophobic or poorly water soluble drugs. In short emulgels are the combination of emulsion and gel.

Advantages of emulgel

1. Increased patient acceptability.

2. Provide targeted drug delivery.

3. Easy termination of the therapy.

4. Improve bioavailability and even the low doses can be effective in comparison with other conventional semi solid preparation.

5. Stable formulation by decreasing surface interfacial tension resulting in increase in viscosity of aqueous phase, more stable than Transdermal preparations that are comparatively less stable, powders are hygroscopic, creams shows phase inversion or breaking and ointment shows rancidity due to oily b

Disadvantages

1. Poor absorption of macromolecules.

2. Entrapment of bubble during formulation.

3. Hydrophobic drugs are the best choice for such delivery systems.

4. skin irritation or allergy reaction on contact dermatitis.

5. Can be used only for drugs which require very small plasma concentration for action.

6. Enzyme in epidermis may denature the drugs

Emulsions are of different types depending on the size of droplets or nature of distribution

1) Macroemulsion gel

2) Nanoemulsion gel

3) Microemulsion gel

1) Macroemulsions gel

These are most common type of emulgels where the particle size of droplets of emulsion is more than 400nm. They are visually opaque but the individual droplets can be easily observed under microscope. Macroemulsion are thermodynamically unstable, but can be stabilized using surface active agents.

E.g. Khullar R. et al, mefenamic acid emulgel was prepared using Carbopol 940 as gelling agent. Liquid paraffin was used as oil phase. Mentha oil and clove oil was used as penetration enhancer. Then it was evaluated for rheological studies, spreading coefficient studies, skin irritation test, in-vitro release, etc.

2) Nanoemulsion gel

When nanoemulsion is incorporated into gel it is called as nanoemulgel. Nanoemulsion are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and co surfactant molecules having a droplet size of less than 100nm. Nanoemulsion formulations possess improved Transdermal and dermal delivery properties in vitro as well as in vivo. This has improved transdermal permeation

of many drugs over the conventional topical formulations such as emulsions and gels. E.g. Singh B. P et al, prepared Carvedilol nanoemulgel using oleic acid and isopropyl myristate (3:1) as oil phase. Tween 20 and Carvedilol were used as surfactant and co surfactant respectively. Carbopol 934 was used as gelling agent.

3) Microemulsion gel:

Microemulsions are transparent and thermodynamically stable as their droplet size range from 10 to 100nm and they do not coalesce. Microemulsions arecomposed of oil, co-surfactant, and water in specific proportions. The ingredients of Microemulsion could facilitate the permeation rate of the drug by reducing the diffusion barrier of the stratum corneum. However, due to low viscosity of Microemulsion, their less retention capacity in the skin restrains its application in the pharmaceutical industry To overcome this disadvantage, gelling agents such as Carbopol 940, xanthan gum and carrageenan have been added into the Microemulsion for forming Microemulsion based gel in order to increase its viscosity which could be suitable for topical application. Moreover, Microemulsion based gel prevents the absorption of drug in the blood stream and provide higher drug accumulation in the skin for efficient action. E.g. Bachhav Y. G et al, prepared clotrimazole Microemulsion based vaginal using Carbapol 90 as oil phase and Cremophor EL as surfactant. Carbopol ETD 2020 is used as gelling agent.

Formulation considerations

The challenges in formulating the topical emulgels are

 Determining systems that are non-toxic, non-irritating, non-comedogenic and non-sensitizing.
 The emulgel formulation must have good physiologically compatable and high biocompatibility.
 Formulation cosmetically elegant emulgel.

Formulation of emulgel includes

1. **Vehicle**
2. Properties of vehicles is that
3.

    Deliver the drug to target site.

    Sustain a therapeutic drug level in the target tissue for a sufficient duration to provide a pharmacological effect.

    Release the drug so it can migrate freely to the site of action.

a) Aqueous material

This forms the aqueous phase of the emulsion. Commonly used agents e.g. water, alcohols.

b) Oils

These agents from the oily phase. For externally applied emulsions, mineral oils, either alone or combined with soft or hard paraffin's are widely used. In oral preparations non-biodegradable mineral and castor oils that provide a local laxative effect and fish liver oils or various fixed oils of vegetable origin (e.g.: arachis, cottonseed, and maize oils) as nutritional supplements.

2) Emulsifier

Emulsifying agents are used both to promote emulsification at the time of manufacture and to control stability during a shelf life. e.g.: Polyethylene glycol 40 stearate, Sorbitan mono-oleate (Span 80), Polyoxyethylene Sorbitan monooleate (Tween 80), Stearic acid, Sodium stearate.

3) Gelling agent

These are used to increase consistency of dosage form and provide gelled behavior. Gelling agent are of 2 types natural and synthetic. Incorporation of gelling agent to a system makes it thixotropic. It is observed that there exist an inverse relationship between concentration of gelling agent and extent of drug released. Then get hydrated and swell. Besides its hydrophilic nature, its cross-linked structure and its insolubility in water makes carbopol a potential candidate for use in controlled release drug delivery system. HPMC emulgel shows better drug release than carbopol. Ex: carbopol-934(1%), HPMC-2910(2.5%).

4) Preservatives: E.g: Propyl paraben, methyl paraben.

5) Antioxidants: E.g: Butylated Hydroxyl Toluene(BHT), Ascorbyl palmitate, Butylated hydroxyanisole(**BHA), etc.**

6) Humectants: to prevent loss of moisture. E.g. Glycerin, Propylene gylcol, etc.

7) Gelling agents: These are the agents used to increase the consistency of any dosage form can also be used as thickening agent. E.g. Carbapol 934, carbapol 940, HPMC, HPMC-2910,sodium CMC.

8) Permeation enhancer: These are agents that partition into and interact with skin constituents to induce a temporary and reversible increase in skin permeability. E.g: Oleic acid, lecithin, isopropyl myristate, urea, eucalyptus oil, chenopodium oil, pyrrolidone, laurocapran, dimethyl sulphoxide, linoelic acid, menthol.

Properties of penetration enhancer

- They should be non-toxic, non-irritating and non-allergenic.
- They would ideally work rapidly and the activity and duration of effect should be both predictable and reproducible.
- They should have no pharmacological activity within the body i.e. should not bind to receptor sites.
- The penetration enhancers should work unidirectional i.e. should allow therapeutic agents into the body while preventing the loss of endogenous material from the body.
- The penetration enhancers should be appropriate for formulation into diverse topical preparations, thus should be compatable with both excipients and drugs.
- They should be cosmetically suitable with an appropriate skin ‘feel’.

Mechanism of penetration enhancer

Penetration enhancers may act by one or more of three main mechanisms

1. Disruption of the highly ordered structure of stratum corneum lipid.

2. Interaction with intercellular protein.

3. Improved partition of the drug, co-enhancer or solvent into the stratum corneum.

4. Most of the hydrophobic drugs cannot be incorporated directly into gel base because solubility act as a barrier and problem arises during the release of the drug.

5. Emulgel helps in the incorporation of hydrophobic drugs into the oil phase and then oily globules are dispersed in aqueous phase resulting in o/w emulsion and this emulsion can be mixed into gel base. This may be proving better stability and release of drug than simply incorporating drugs into gel base.

Method of preparation

It consist of 3 steps

Step-1: formulation of emulsion either o/w or w/opreparation of oil phase: oil phase of the emulsion is prepared by dissolving emulsifier.

E.g: span 20 in oil phase lie light liquid paraffin.Preparation of aqueous phase: aqueous phase is prepared by dissolving emisifier. E.g:tween 20 in purified water.

Step-2 : formulation of gel base: prepared by dispersing polymer in purified water with constant stirring at a moderate speed using mechanical shaker, then the pH was adjusted to 6-6.5 using tri ethanolamine (TEA). **Step-3 :** incorporation of emulsion into gel base: Add glutaraldehyde in during mixing of gel and emulsion in ratio of 1:1 to obtain emulgel.

The main aim of the present study was to formulate the Annona Squamosa leaf extract emulgel so as to rerain the formulation for prolong period of time and delivery of the drug to desired site of absorption.To overcome the limitations of hydro gels in the delivery of hydrophobic drugs. To evaluate and formulate the Annona Squamosa leaf extract topical emulgel. To develop an emulsion based gel formulation for a antimicrobial, antifungal infections. To overcome the limitations of hydro gels in the delivery of hydrophobic drugs.

METHODOLOGY

A) Preparation of leaves extract Annona squamosal

B)

The collected fresh leaves of Annona squamosa were washed with water and dried in shade. After drying plant leaves were coarsely powderd and kept in well closed container. About 100gm of coarse powder of leaf was weighed and soaked in 500 ml of ethanol andleft for maceration for about 4-5 days. After maceration the extract was concentrated and used for further formulations.

Formulation

1. Preparation of Emulsion

A) Preparation of Aqueous Phase

The aqueous phse of the emulsion was prepared by dissolving tween 80 in purified water.

B) Preparation of oil Phase:

Methyl parabeen and propyl paraben were dissolved in propylene glycol where as drug was dissolved in ethanol and both solution were mixed withaqueous phase. Both the oily and aqueous phase were separately heated to 75◦c. then the oil phase was added to the aqueous phase with continous stirring until cooled to room temperature.

2. Preparation of gel:

The gel bases were prepared by dispersing different concentrations of polymers in distilled water separately with constant stirring at a moderate speed using mechanical shaker. The pH of all formulations was adjusted to 6 - 6.5 using triethanolamine (TEA).

3. Preparation of Emulgel:

The obtained emulsion was mixed with the gel with gentle stirring to obtain the emulgel.

Table 1 : Preparation of Emulgel

Ingredients	F1	F2	F3	F4	F5
Annona squamosa leaf Extract	0.5gm	1.0gm	1.5gm	2.5gm	3.0gm
Carbopol 940	1.0gm	1.0gm	1.0gm	1.0gm	1.0gm

Xantham gum	1.0gm	1.0gm	1.0gm	1.0gm	1.0gm
Propylene glycol	10ml	10ml	10ml	10ml	10ml
Methyl paraben (0.5 %)	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Propyl paraben (0.2 %)	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
Glycerine	1ml	1ml	1ml	1ml	1ml
Triethanolamine (to adjust pH)	q.s	q.s	q.s	q.s	q.s
Distilled water	100ml	100ml	100ml	100ml	100ml

Evaluation Parameters of ornidazole Emulgel

Physical appearance: The prepared Emulgel is checked visually for their color, homogeneity, consistency and phase separation.

PH Evaluation: pH evaluation is the important criteria especially for the topical formulation.The pH of emulgel should be between 5.8 – 6 to mimic the skin condition. If the pH of the prepared emulgel is acidic or basic, it may cause irritation to the patient. PH of the prepared emulgel was measured using digital pH meter by dipping the glass electrode into an emulgel. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Spreadability: Spreadability of emulgel is measured in terms of diameter of emulgel circle produced when emulgel is placed between two glass plates of definite weight. A weighed quantity (350 mg) of emulgel is taken on one glass plate and another glass plate is dropped from a distance of 5 cm. The diameter of the circle of spread emulgel is measured.

It is calculated by using the formula:

S=M.L/T

Where,

S= spredability

M= weight tied to upper slide.

L= length of glass slide.

T= time taken to separate the slides completely.

Skin Irritation Test (Patch Test): For this study emulgel is applied on the shaven skin of rat and its adverse effect like change in color, change in skin morphology are evaluated up to 24 hours. About 8 rats can be used for the study. Test passes if no irritation shown. If it fails the test is repeated with another 2 rats.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu,IR, Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4 cm -1

Differential Scanning Calorimetry Analysis:

The DSC was used to measure the occurrence of exothermic or endothermic changes with increase in temperature. The DSC because of its sensitivity and accuracy has been extensively used to study the phase transition of polymer. Differential Scanning Calorimetry (DSC) measures the temperature and heat in the material. It determines time function and temperature in a controlled atmosphere. These measurements provide quantitative and qualitative information about physical and chemical changes that involve during endothermic or exothermic processes, or changes in heat capacity. The onset, peak and conclusion temperatures of base transition were observed to be moderate. The knowledge of glass transition temperature is essential in production processes and storage glass transition temperature is affected by moisture and other additives, facilitating conversion to the rubbery state and hence fasciltating crystallization through molecular rearrangement. Procedure:

To carry out DSC testing a required quantity of sample was taken. Then this sample was placed in the pan. This pan is carefully handled by using forceps. This was covered by using cap and pressed well. After this crucible is kept in the instrument where it comes in between length path and graph is obtained. The x-axis shows the time and energy change is given on y-axis. The DSC of synthesized silver nanoparticles in are shows in figure.

Extrudability Study [tube test]: It is calculated by the force required to extrude the emulgel from the tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow. In this study emulgel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5cm ribbon of emulgel in 10 seconds. For better extrudability, more quantity is extruded. For the measurement of extrudability, it is done in triplicate and the average values are calculated. The extrudability is then calculated by using the following formula.

Extrudability = weight applied to extrude emulgel from tube (in gm) / Area (in cm^2).

Rheological Studies: Viscosity of emulgel is determined at 25°C using a cone and plate viscometer with spindle

52 and connected to a thermostatically controlled circulating water bath.

Swelling Index: It is determined by taking 1g of emulgel in a porous aluminum foil and mixed with 0.1N NaOH kept in a 50ml beaker. Then samples are withdrawn at different time intervals and kept for drying and it is reweighed. Swelling index is calculated as follows:

Swelling Index = {Wt – Wo/Wo}100

Where,

(SW) % = Equilibrium percent swelling,

Wt = Weight of swollen emulgel after time 't'

Wo = weight of emulgel at zero time.

Drug Content Determination

Emulgel is mixed in a suitable solvent. Filter it to obtain clear solution. Determine itsabsorbance using UV spectrophotometer. From the standard equation by putting theabsorbance value concentration and drug content can be obtained.

Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor.

Bioadhesive strength measurement

The modified method was used for the measurement of bioadhesive strength. The apparatus consist of two arm balance, both the ends are tied to glass plates using strings. One side contains single glass plate for keeping weight. The right and left pans were balanced by adding extra weight on the left hand pan. The balance was kept in this position for 5 mints.

Procedure: Accurately weighed 1g of emulgel was placed between these two slides containing hair less fresh rat skin pieces, extra weight from the left pan was removed to sandwitch the two pieces of glass and some pressure was applied to remove the presence of air. The balance was kept in the position for 5 min. weight was added slowly at 200mg/min to the left hand pan until the two glass slides got detached from each others. The weight required to detach the emulgel from the glass surface gives the measure of bioadhesive strength by using a formula,

Bioadhesive strength=weight required (in g) / area(cm2)

Microbiological assay: Microbiological assay was performed by using ditch plate technique. Previously prepared Sabouraud's agar dried plates were used. 3 grams of the gellified emulsion are placed in a ditch cut in plate. Freshly prepared culture loops are streaked across agar at a anglefrm the edge of the plate. After incubation for 18-24hrs at 25◦c, the fungal growth was observed and the percentage inhibition was measured as follows

% inhibition = L2 /L1× 100

In-vitro Release Studies: The in vitro drug release studies were carried out using a modified franz diffusion (FD) cell the formulation was applied on dialysis membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer PH 7.4 was used as a donor and receptor compartment of the cell was maintained at 37c by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar bank set was run simultaneously as a control.sample (5 ml) was withdrawn at suitable time intervals and replaced with equal amount of fresh dissolution media. Samples were analysed spectrophotometrically at 318nm and cumulative %drug release was calculated

Ex Vivo Release Study

The ex vivo drug release study of selected formulations (F2and F4) was carried out in a modified Franz diffusion cell, using wistar male rat skin. A section of skin was cut and placed in the space between the donor and receptor compartment of the FD cell, keeping the dorsal side upward. Phosphate buffer pH 7.4 was used as dissolution media. The temperature of the cell was maintained constant at 32 -C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar blank set was run simultaneously. The samples were withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media (Schreier and Bouwstra, 1985). Samples were analyzed spectrophotometrically at 285 nm.

Stability studies

The prepared emulgels were packed in aluminum collapsible tubes (5 g) and subjected to stability studies at 5 -C, 25 -C/60% RH, 30 -C/65% RH, and 40 -C/75% RH for a period of 3 months. Samples were withdrawn at 15-day time intervals and evaluated for physical appearance, pH, rheological properties and drug content (Harmonized Tripartite Guidelines,2003).

RESULT AND DISCUSSION:

Evaluation of prepared formulation

The topical gels were prepared and subjected to physical evaluations such as appearance, pH, viscosity, spreadability and skin irritation. The gels were clear throughout the evaluation. The pH was constant (6.8 – 7.0) and did not produce any type of irritationwhen applied on the skin. Viscosity, and spreadability were excellent.

Emulgel formulations were light green viscous creamy preparation with a smooth homogeneous texture and glossy appearance. Results have been discussed in Table 2.

Table 2: Physical parameters of formulation batches.

Formulation Code	Appearance	Phase Separation	Grittiness	Homogenicity	Consistency
F1	Light green	None	-	+++	+++
F2	Light green	None	-	+++	+++
F3	Light green	None	-	+++	+++
F4	Light green	None	-	+++	+++
F5	Dark greenish	None	-	+++	+++

Measurement of pH

Table 3: Measurement of pH

Sr No	Formulation	pH
1	F1	6.8
2	F2	6.8
3	F3	6.9
4	F4	6.8
5	F5	7.0

Spreadability Coefficient

The spreading coefficient of various emulgel formulations are given below in Table 4

Table 4: Spreadability

Sr No	Formulation	Spreadability (g.cm/sec)
1	F1	15.65
2	F2	13.45
3	F3	12.36
4	F4	14.60
5	F5	13.00

Rheological Studies

The tests were performed at 100 rpm for 10 min. Results are given in Table 5

Table 5: Rheological Studies

Sr No	Formulation	Viscosity (Centipose×10^3)
1	F1	79
2	F2	82
3	F3	85
4	F4	81
5	F5	84

Sweeling Index

The swelling index of different formulations was observed and the data was shown in Table n.o. 12.

Table 6: Sweeling Index

Time (min)	F1	F2	F3	F4	F5
0	1	1	1	1	1
15	1.15	1.12	1.22	1.13	1.15
30	1.10	1.14	1.30	1.19	1.17
45	1.20	1.10	1.16	1.1	1.15
60	1.4	1.22	1.29	1.25	1.20
120	1.2	1.8	1.98	1.26	1.23

Determination of Drug Content

Drug content of each formulation was determined and data was shown in table n.o As shown in the table of drug content uniformly. The drug content uniformity of all the formulations was found to be ranging from 80% to 95% was observed that as the concentration of emulsifying agent increasing the drug content increased.

Table 7: Drug Content

Sr No	Formulation	Drug Content
1	F1	89%
2	F2	86%
3	F3	95%
4	F4	92%
5	F5	94%

Skin Irritation

No allergic symptoms like inflammation, redness, irritation appeared on rats up to 24 h.

Table 8: Skin Irritation

Sr No	Formulation	Skin Irritation
1	F1	nil
2	F2	nil
3	F3	nil
4	F4	nil
5	F5	nil

Bioadhesive Strength Measurement

The bioadhesive strength of emulgel formulations have been shown below in Fig. 1

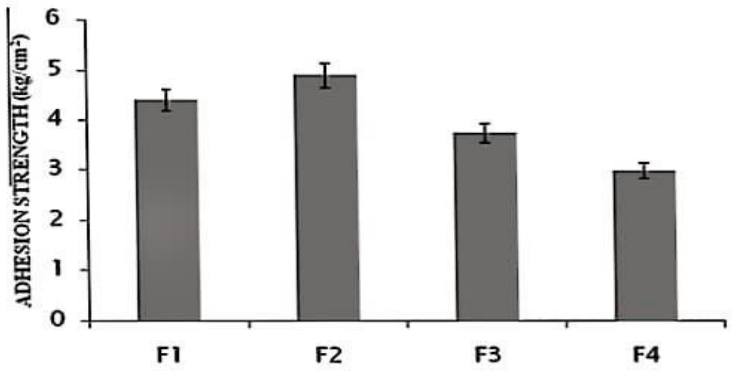


Fig 1: Bioadhesive Strength Measurement

Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the compoud. The FTIR spectrum of annona squamosa leaf extract ,natural geling agent carbapol 940 and xantham gum in case showed the band between 3490-3500 cm-1corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm-1showed a stretch forC-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N-H. C=O showed the bond stretch for 1730-1750 cm-1.Whereas the stretch for were found around 500-550 cm-1From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that extract to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of in the aqueous medium. Flavanones or terpenoids could

be adsorbed on the surface of, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong legating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation. These issues can be addressed once the various fractions of the Annona Squamosa leaf extract are separated, identified and individually assayed for reduction of the metal ions..

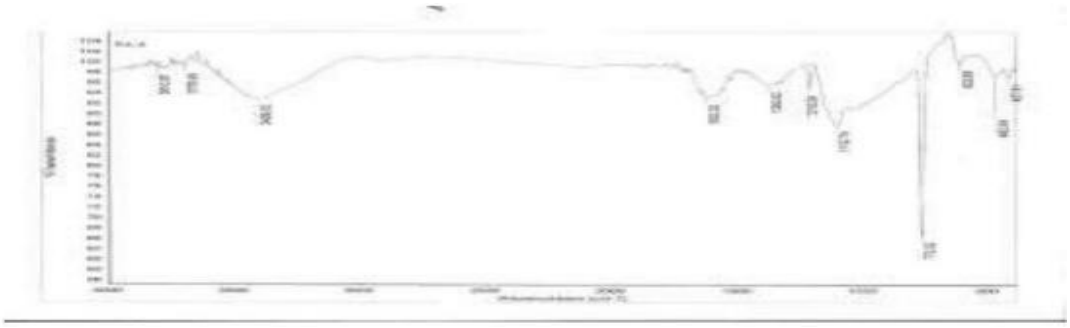


Fig 2: FTIR of Annona Squamosa leaf extract

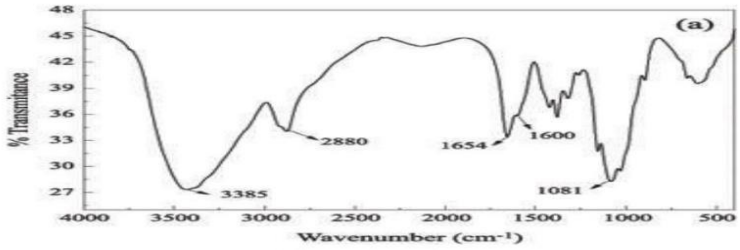


Fig 3: FTIR of Carbapol 940

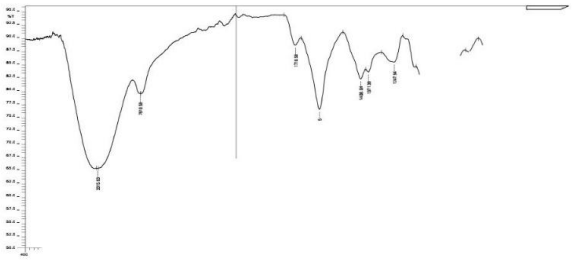


Fig 4: FTIR of Annona Squamosa leaf extract Carbapol 940+Xanthum gum gel formulation

Differential Scanning Calorimetry Analysis:

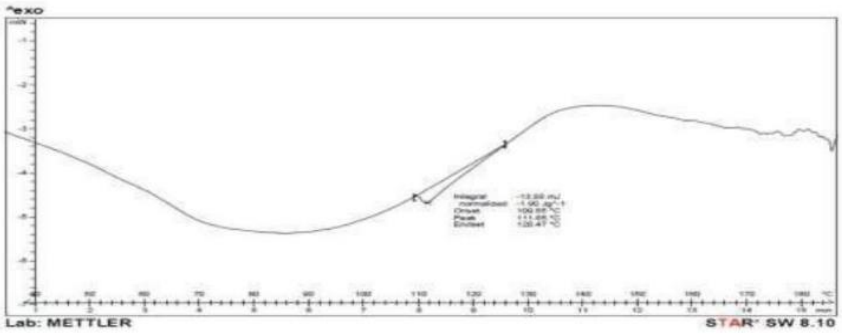


Fig 5: DSC of Annona Squamosa leaf extract

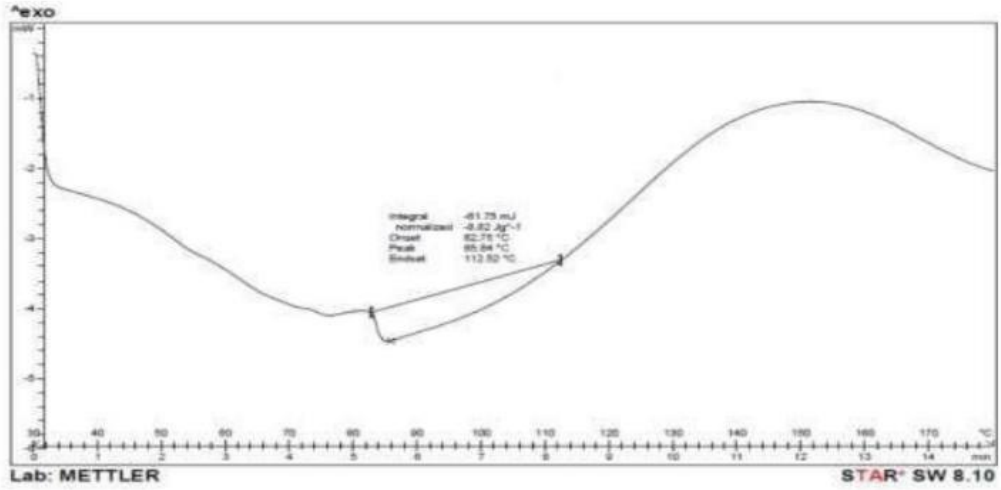


Fig 6: DSC of Annona Squamosa leaf extract Carbapol 940+Xanthum gum gel formulation

In the above fig.t DSC of Annona Squamosa plant extract graph shows melting temperature i.e. onset-109.65˚C, Peak-111.65˚C & Endset-120.47˚C which is maximum temperature at which extract degraded. And in the fig.6 DSC graph Annona Squamosa leaf extrac+Carbapol 940 +Xntham gum gel formulation extract shows melting temperature i.e. onset-82.75˚C Peak-85.84˚C & Endset-112.52˚C which is maximum temperature.

Table 9: DSC Parameter of Annona Squamosa leaf Extract

Sr No.	**Parameter**	**Annona Squamosa leaf Extract**	**Annona Squamosa leaf extract +Carbapol 940 +Xantham gum gel formulation**
1	Onset Temperature (˚C)	109.65˚C	82.75˚C

2	Peak Temperature (˚C)	111.65˚C	85.84˚C
3	Endset Temperature (˚C)	120.47˚C	112.52˚C

Microbiological assay

The results of antibacterial activity of all formulated herbal gels against some pathogenic microorganisms is shown in below Table 6

Fig 7: Antibacterial activity against Escherichia coli

Fig 8: Antibacterial activity against Bacillus subtilis.

Fig 9: Antibacterial activity against Streptococcus aureus

Table 10: Antibacterial activity of herbal gels

Micro-organism culture	Zone of inhibition of Herbal gels (mm)					
	Standard drug	F1	F2	F3	F4	F5
S. aureus	28	11	14	17	21	25
E. coli	30	10	13	16	20	24
B. subtilus	36	12	18	24	28	32

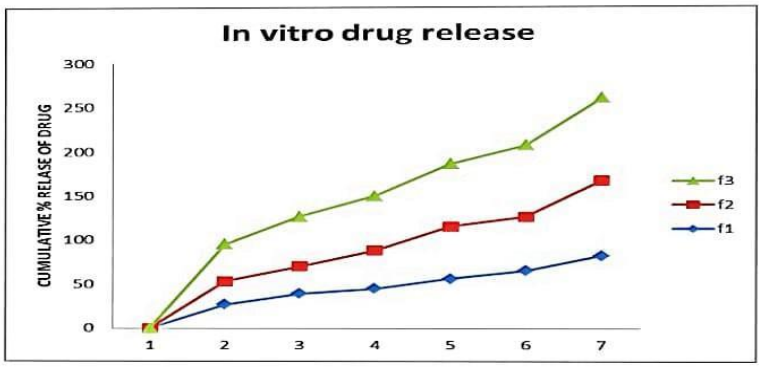
In Vitro Drug Release

The cumulative % drug release profile of the formulation batches has been shown in Table- and as shown in table of in vitro release studies, the release profile increases with increase in the concentration of emulsifier. From the emulgel formulations (F3) showed high amount of drug release. i.e. (92%) in 6 hrs. in vitro cumulative % drug release data of formulations was shown in table n.o 11

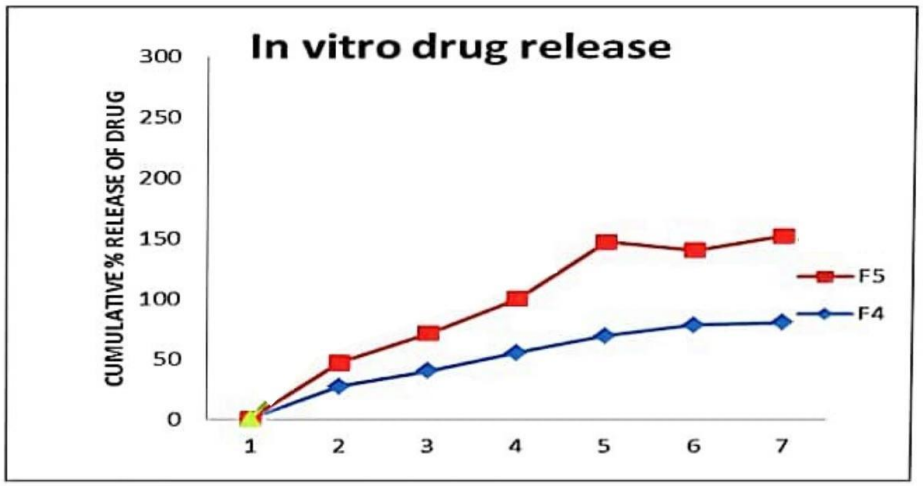
Table 11: % Drug Release Data of Formulations

Time (min)	F1	F2	F3
0	0.00	0.00	0.00
60	27	26	42.25
120	39	31	56.83

180	45	43	61.58
240	56	55	71.00
300	65	64	81.80
360	82	74	93.20



Time (min)	**F4**	**F5**
0	0.00	0.00
60	27	20
120	40	31
180	55	45
240	69	78
300	78	62
360	80	72



Ex Vivo Release Study

This study was carried out only on two best optimized formulations. The study showed the release of the drugs from its emulsified gel formulation F2 and F4 were 55.47% and 56.60%, respectively in 240 min. The results are show in Fig. 5.

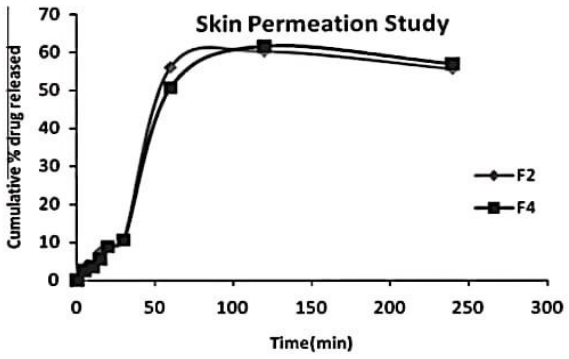


Fig 10: Ex vivo cumulative % release of formulations F2 and F4

Stability study

All the prepared emulgel formulations were found to be stable upon storage for 3 months, no change was observed in their physical appearance, pH, rheological properties and drug content.

CONCLUSION

The present study was to increase the penetration of the drug into the skin. In coming years, the topical drug delivery will be used extensively to improve better patient compliance. In present study Annuna squamosa leaf extract topical emulgels were prepared by using different polymers such as Carbapol 940, Xanthum gum,

Propylene glycol, Methyl paraben, Propyl paraben, Glycerine, Triethanolamine (to adjust pH), and Distilled water. These emulgel are used for the treatment of Psorasis.The colour of the formulated herbal emulgel was Light greenish to dark greenish and all the herbal emulgel were good in homogeneity. The pH of the formulated emulgel was in the range of 6.4- 7.1 matching with skin pH range. Viscosity of the herbal gels was ranging from 79- 84 cp x 103 at 100 rpm measured with Brookfield viscometer. The spreadability of herbal emulgel was in the range of 12.36-15.65 (g.cm/sec). The antibacterial activity of the formulated herbal gels showed good results of zone of inhibition against skin pathogens.In this study all the formulations were subjected to various evaluation parameters such as physical appearance, pH evaluation, spreadability, swelling index, Rheological studies, FTIR, DSC, Microbiological assay, drug content, in vitro drug release, ex vivo release study and stability study were found to be within the limits\among all the formulations formulated Annuna squamosa leaf extract emulgel.

REFERENCES

1) http://www.sign.ac.uk/pdf/pat121. PD
2) Menter A, Korman NJ, Elmets CA, et al. (2009) American Academy of Dermatology. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. J Am Acad Dermatol. 60(4):643-659.
3) Menter A, Korman NJ, Elmets CA, et al. (2010) Guidelines of care for the management of psoriasis and psoriatial arthritis: Section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy. J Am Acad Dermatol.; 62(1):114-135
4) Chalmers RJ, O'Sullivan T, Owen CM, Griffiths CE. (2001) A systematic review of treatments for guttate psoriasis. Br J Dermatol; 145(6):891-4.
5) Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Heading ton JT, et al. Cyclosporine improves psoriasis double blind study. JAMA 1986; 256:3110-6
6) Toichi E, Torres G, McCormick TS, Chang T, Mascelli MA, Kauffman, CL, et al. An anti-IL- 12p40 antibody down-regulates Type 1 cytokines, chemokines, and IL-12/IL-23 in psoriasis. J Immunol 2006; 177:4917-26
7) Sarac G, Kocal TT, Baglan T. A summary of clinical types of psoriasis. North Clin Istanbul 2016; 3:79-82.
8) Khandelwal K.R. Practical pharmacognosy Techniques and experiments. 9th edition. Pune, Nirali Prakashan; 2002: 149-160.
9) Ayurvedic Pharmacopoeia, 1st edition. Government of India. Ministry of health and family welfare department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy, New Delhi, 2007; 3: 25-26.

10) Singh M.K., Khare G., Iyer S., Sharma G., Tripathi D.K. Clerodendrum serratum: A Clinical approach. JAPS. 2012: 2(2): 11-15.
11) Niyogi P., Raju N.J., Reddy P.G., Rao B.G. Formulation and evaluation of anti inflammatory activity of Solanum Pubescens Wild extracts gel on albino Wistar rats. Int.J.Pharm.2012: 2 (3): 484-490.
12) Mishra U.S., Murthy P.N., Pasa G., Nayak R.K. Formulation and evaluation of herbal gel containing methanolic extract of Ziziphus Xylopyrus. IJBPR.2011: 1(4): 207-218.
13) Rajesh B, Saumya D, Dhramjit P, Pavani M. Formulation design and optimization of herbal gel containing Albizia lebback bark extract. Int J Pharm Pharm Sci. 6 (1); 2014: 111-114.
14) Salgaonskar Snehal and Padaila Unnati . Development of anti-fungal herbal hand wash gel, Int. J. of Life sciences, Special Issue, A5:86-88.
15) Sandeep D. S*, Prashant Nayak, Jobin Jose, Rishal Relita M, Sumana D. R. Formulation and evaluation of antibacterial herbal gels of Murraya koenigii leaves extract. Res J Pharm Tech., 2017; 10(6): 1-2.
16) Dixit G., Misal G., gulkari V., Upadhye K. Formulation and evaluation of polyherbal gel for anti-inflammatory activity. IJPSR.2013: 4(3): 1186-1191.
17) Kulkarni . V. R, Chandershekhar. C. Isolation,Characterizations and Free radical scavenging activity of Annona squamosa leaf,Journal of Pharmacy Research 2011,4(3),610- 611.
18) Goyal S., et al. "Novel Anti-Inflammatory Topical Herbal Gels Containing Withania somnifera and Boswellia serrata". International Journal of Pharmaceutical and Biological Archives 2.4 (2011): 1087-1094.
19) Rajesh B, Saumya D, Dhramjit P, Pavani M. Formulation design and optimization of herbal gel containing Albizia lebback bark extract. Int J Pharm Pharm Sci., 2014; 6(1): 111-114.
20) Leelaprakash G., Das S.M. Invitro anti inflammatory activity of methanolic extract of Enicostemma Axillare. IJDDR.2010: 3(3): 189-196.
21) Jadhav VD, Talele SG, Bakliwal AA, Chaudhari GN. Formulation and evaluation of herbal gel containing Tridax procumbens. J Pharma Bio Sci., 2015; 3(2): 165-172.
22) Ajinkya MB, Manjusha ND. Formulation and evaluation of herbal antimicrobial gel containing Musa acuminata leaves extract. J Pharamcog Phytochem, 2016; 5(1): 1-3.
23) Vidya V, Aleykutty NA, Jayakar B, Subin MZ. Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of Samadera indica for skin diseases. J Adv Pharm Technol Res., 2012; 3(2): 106-111.
24) Hugo WB, Russell AD. Pharmaceutical Microbiology. Oxford, UK:Blackwell Scientific Publications; 1977:190.
25) Rathbone MJ, Hadgraft J, Roberts MS, Lane ME. Dermal and transdermal drug delivery.
26) Modified –release drug delivery technology, 2nd ed. U.K: Informa healthcare, 2002; 263-71.

27) Ajazuddin, Alexander A, Khichariya A, Gupta S, Patel RJ, Giri TK et al. Recent Expansions in an emergent novel drug delivery technology: Emulgel. J Control Release,2013; 171: 122-32.

28) Bhowmik D, Gopinath H, Kumar PB, Duraivel S, Kumar KPS. Recent advances in novel topical drug delivery system. Pharma innovation, 2012; 1: 12-31.

29) R. P. Singh*, S. Parpani, R. Narke, R. Chavan: Emulgel: A Recent Approach For Topical Drug Delivery System, Asian Journal of Pharmaceutical Research and Development,March –April. 2014; 2(2): 112-123.

30) Mohamed, S. D. (2004). Optimization of ChlorphenesinEmulgel Formulation. American Association of Pharmaceutical Scientists, 2004; 6(3): 1-7.

31) Anu Hardenia, Sonali Jayronia and Sanjay Jain: Emulgel: An Emergent Tool In Topical Drug Delivery, Ijpsr 2014; 5(5).

32) Gaur PK, Mishra S, Purohit S and Dave K: Transdermal Drug Delivery System: A Review. Asian Journal of Pharmaceutical and Clinical Research, 2009.

33) Bachhav Y., Patravale V. Microemulsion based vaginal gel of fluconazole: formulation, in vitro and in-vivo evaluation. Int J Pharmaceutics, 2009; 365: 175-179.

34) Baibhav J., Rana G., Vikas S. Comprehensive review on the recent advances in topical Drug delivery. Int Res J Pharm, 2011; 66-70.

35) Cecv G. Preclinical characterisation of NSAIDS in ultra deformable carriers or Conventional topical gels. Int J Pharm, 2008; 1520-1621.

36) Chaudhary A., Tiwari N., Jain V., Singh R. Microporous bilayer osmotic tablet for colon Specific delivery. Eur J Pharmaceut Biopharmaceut, 2011; 78(1): 134-140.

37) Kumar L, Verma R, In vitro evaluation of topical gel prepared using natural polymer. Int J drug delivery, (2010).

38) Mitkari BV, Korde SA, Mahadik KR, Kokare CR, Formulation and evaluation of topical liposomal gel for Fluconazole. Indian journal of pharmaceutical education and research,2010; 44(4): 324-329.

39) Kshirsagar N A. Drug Delivery Systems. Ind. J. Pharmacol, 2000; 32: S54- S61.

40) Rashmi M. Topical gel: A review august vol. 2008; available from

41) http://www.pharmainfo.com