Original Article

Herbal Based Nanogel Formulation For Skin Disease -Optimization And Evaluation Parameters

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DOI: 10.47750/pnr.2023.14.03.363

Abstract

Skin diseases are a common problem faced by people worldwide, and there is a growing demand for natural and safe solutions to treat them. Nanogels are a promising drug delivery system due to their small size, high surface area, and ability to encapsulate both hydrophilic and hydrophobic compounds. In this study, we aimed to optimize and evaluate the parameters of a herbal-based nanogel formulation for the treatment of skin diseases. The nanogel formulation was prepared using a combination of natural polysaccharides, namely chitosan and sodium alginate, and a herbal extract of neem leaves. The optimization parameters included varying the concentrations of chitosan, sodium alginate, and neem extract, and the pH of the formulation. The optimized formulation was evaluated for its physicochemical properties, drug release profile, stability, and skin irritation potential. The results showed that the optimal nanogel formulation had a particle size of 200 nm, a zeta potential of +25 mV, and a drug entrapment efficiency of 80%. The drug release profile was found to be sustained, with 60% of the drug being released over 8 hours. The stability studies showed that the formulation was stable for 3 months at 4°C. The skin irritation studies indicated that the nanogel formulation was non-irritating and safe for topical application.
The herbal-based nanogel formulation developed in this study has the potential to be a safe and effective treatment option for skin diseases. Further studies are required to investigate its efficacy in vivo and in clinical trials.

KEYWORD: Herbal Medicinal Plants, Byproducts, Protein Denaturation, Antigen.

INTRODUCTION

In recent years, the use of herbal medicinal plants has gained fame in primary health care needs during the world including developing countries. Medicinal herbal plants are the spine of old medical practice, as most contemporary drugs are formed from these plants and their byproducts [1].

Inflammation is body's defense system and helps in healing process. It is complex biological response which involves increase in vascular permeability, protein denaturation and membrane alteration. When body detects any foreign antigen then defense mechanism gets triggered and started its biological response, such as rashes, pain, swelling & dysfunction of injured part. Mainly burns, trauma, infections and many types of auto immune response occur due to inflammation [2].

There are several routes is used for the drug administration, but the most common route is oral administration but via this route only 50 percent of drug can be administrated because due low solubility in water. The low solubility drug shows low dissolution rate in the gastrointestinal fluid that results low bioavailability [3-4]. Whereas, bioavailability is describe in Biopharmaceutics Classification system commonly known as BCS, the drug comes under Class II categories simply considered as increase in bioavailability when solubility and dissolution rate is increased in gastrointestinal fluids [5-6]. The uptake of poorly soluble drug with slow dissolution rate cannot be completed within

the time at absorption site & leads to decomposition of drug in gastrointestinal tract. To avoid this, Self-emulsifying delivery system is new promising approach has been developed to improve the bioavailability of the drug. This system helps in reducing the slow & incomplete absorption, increases the transportation via lymphatic system and absorption from GIT [7-8].

In recent studies, an herbal drug has proved an immense success, in this one of inflammation related to skin diseases. The single herbal drug i.e. Aegle marmelos, which is slow growing tree having short trunk, soft and spiny branches occasionally. This is herbal plant is used to treat different diseases like inflammation, diabetes, anticancer, peptic ulcer, dysentery, cholesterol, diarrhea, cardioprotective, antibacterial, antifungal, radioprotective , antipyretic, analgesic, constipation , respiratory infection, antioxidant, hepatoprotective and wound healing [9].

Another example of single herbal drug that has proved boon in medical science is Emblica officinalis commonly known as Amla belonging to Euphorbiaceae family. Herbal plant have several parts like leaves, fruit, seed, root, flower, bark etc., basically this all parts have some medicinal value which is varies with the plant. In this, Amla have several medicinal properties like analgesic, antioxidant, antipyretic, adoptogenic, immunomodulatory and antiulcerogenic. There are several chemical constituent present in Emblica officinalis such as tannins, polyphenols, flavanoids & high amount of vitamin C [10-21]. Inspite of wide range of activities of A. marmelos and E. officinalis has been used in treatment of inflammation, yet no reports shows topical dosage form of from extract of this plant. Thus, the present study aimed on using the methanolic extract of this plant for the effectiveness of the anti-inflammatory via topical delivery [9].

There are many herbal plants that are broadly used for the Skin diseases like rheumatoid arthirits & osteoarthritis for therapeutic purposes, as plants are considered to be the lowermost in toxicity, cheap and cause less adverse side effects linked to allopathic or synthetic drugs (Stavric, 1994) [22]. Medicinal plants hence play an significant role in the treatment skin such as inflammation, especially in developing nations where plants are readily obtainable [23].

The idea of polyherbal formulation has been well familiar in antique literature. Old-style medicinal systems typically assume that the synergy effects of all plant components will carry extreme therapeutic effectiveness[24].Herbal combinations are more effective than individual elements of one plant .The polyherbal formulation has better and extended therapeutic potential associated to the single herb, improves the therapeutic action and reduces concentrations of single herbs, thus reducing adverse events[25].The synergistic connections between the constituents are thought to be accountable for therapeutic effectiveness [26-27]. The intake of drug by oral route may causes various problems such as gastrointestinal ulcers and bleeding [28-29]. Because of this, many more serious problems also occurs. So, avoiding these all alternative route has been developed i.e. transdermal system because the previously studied reported that, the herb containing curcumin as active constituent is used in such type of delivery system because it's have a properties to avoid the first pass metabolism, increase patient compliance, reduce or no side effects and increase the duration of time of plasma level in skin [28, 30]. Its active constituent has properties of low penetration to the skin, that's why we develop the formulation in the form of gel or patches. When we use the oil in water formulation this show the high solubility of oil in water which softener the skin [30-31].

Previous study provides the information that microemulsion formulation enhances the topical delivery properties such as in vitro [32-40] and in-vivo [41-43]. Microemulsion also provides better results over the normal topical formulation such as emulsion and gel [44-47].

The herbal plant Eupatorium, belonging to the Asteraceae family, his plant have medicinal value and therapeutically helps in healing, antimicrobial, also treat the fungal disease, inflammation and analgesic effects [48-50]. In the reported study, extracted the leaves of Eupatoriumin ethanolic solvent to perform the anti-inflammatory activity in mouse [51-52].

The another plant Portulaca quadrifida, Portulacaceac family is generally used to treat ulcer, eczema and dermatitis [53-57]. Acne vulgaris is one of the cause related to skin disuses, this could be treated by making the ethanolic extracted of the plant Portulaca quadrifida because this plant have anti-acne and anti-inflammatory properties [58].

Arthritis comes under the autoimmune disorder. For treating the autoimmune disorder generally we suggest the non-steroidal anti-inflammatory drug, immunosuppressant drug, anti-rheumatic, those drugs produce many of side effects such as gastrointestinal and hormonal disturbances. Today scenario , we all approach the drug which have less or no

side effects , than ideas comes our mind why we not use the herbal drug , because these herbal drug comes under the Ayurveda and Sidha system for the treatment of such type of disorder and diseases. As per study Cardiospermum halicacbum used for the treatment of inflammation, rheumatism, analgesic and vasodepressant activities, anti-pyretic activity, anti malarial activity, anti-oxidant activity, anti-ulcer activity. The active compound of this drug is apigenin [59]. Vitex negundo is alo a herbal drug which consists of flavanoids, alkaloids and terpenoids, in this luteolin is compound that have potent anti-arthritic, anti-inflammatory, anti-pyretic. This drug also used as anti bacterial and anti histaminic [60-63].

Both the herb was used to prepare a topical gel formulation for the treatment of arthritis because both drug have anti-arthritic activity. The advantage of this type of formulation is that it is easy to apply, produced localized effect, no skin irritation, no GI disorder or directly applied to the affected area [64].

As per studies, the skin diseases is mostly occurs to the every human being in his our life cycle, from this Acne vulgaris is one of the skin diseases observed by comedones, papules and pustules this comes under inflammatory disease. The possibilities of occurrence to the individual at different percent such as below 25 years occur mostly around 85 percent; adult people affected around 8 percent and approximately 3 percent with the age 35 to 44 years [65-67].

Types of Acne Vulgaris [68]:

1. Infantile acne
2. Acne Conglobata
3. Acne Eexcoriee
4. Acne Fulminance
5. Gram Negative Folliculitis
6. Steroid acne

1. Infantile acne: It is occur due to maternal androgens, observed in face.
2. Acne Conglobata: It is follicular triad type of acne wihich is most severe type.
3. Acne Eexcoriee: Possibility of this type of acne in adult girls and young women, mainly occur to skin observed as lesion.
4. Acne Fulminance: Possibility of this type of acne in adult boys, this is ulcerative type of acne. In this persons losses him weight, having fever, etc.
5. Gram Negative Folliculitis: It is observed in perioral and perinasal area effects with the result of gram negative organism. This could also result with the long term of antibacterial treatment.
6. Steroid acne: This is happen due to cushing syndrome, in this rashes appear on effected area i.e. back and shoulder.

Acne is a skin diseases which is occur in pilosebaceous glands. There are several factors for the occurrence of acne such as:

1. Hormone:
2. Sebum:
3. Bacteria origin:
4. Inflammation:
5. Nutrition:
    1. Hormone: In the adult age generally the hormone dehydrotestosterone increased in our body due to this hyperkeratinisation occurs; which result the infundibular keratinocytes. Due to the occurrence of follicular infundibulum and sebaceous duct, these result acne lesions [69].
    2. Sebum: Secretion of sebum depend on the sebum production, if secretion of sebum increased due to this happen sebaceous gland increases; which stimulated the androgen hormone. By this, Patients is affected by this type of acne that result decrease the level of sebum antioxidant vitamin E & shows lipoperoxide, this is happen due to peroxidation of squalene [67,70].
    3. Bacteria: Propionibacterium acne is anaerobic type of bacteria and S. epidermis is aerobic type of bacteria, these are both are the area for the anti acne drugs. P.acne acts on beneath surface of skin which is accumulate

androgen that astimulated sebaceous follicles. P.acne is best suited for gram positive bacteria. S.epidermis is observed in superficial skin infection with sebaceous [71-72].

4. Inflammation acne: This consists of pustules, papules and nodules [69].
5. Nutrition: IGF-1 level developed in teenage years due to this increase the blood sugar level and serum insulin, this is increasing due to intake milk , due to this high glycemia occurs which resut IGF-1 mediated elevation of steroid. In this acne increases with steroid hormones increases [69,73]

The broad spectrum antibiotic drug is used to treat the fungal infection [74].This drug category is poorly soluble and having serious side effects with less therapeutic effect [75]. To eliminated the toxicity like nephtrotoxicity, locally side effects via topical delivery system with anti fungal drug [76].

The stratum corneum prevents the percutaneous permeability of drug, to break this barrier we use hydrophilic and high molecular weight drug should be used to treat the fungal infection. Here we use AmB because this drug have good carrier for topical delivery, this shows low irritation, enhance permeation and drug loading capacity. The carriers which are used for such type of delivery is Microemulsion, Liposomes and Sold lipid nanoparticle [77-79]. From this system , microemulsion delivery consisted of drug increase the biaovailabilty , stability and lower side effects [80-81] and this system is reduce cost and easy to prepared at industrial level. Drug enhances the localization and penetration within the skin for systemic effectiveness when we applied nano emulsion formulation, this system also increase the permeability of hydrophobic drug. In a particular study, this formulation is performed the in vitro study for check of antifungal activity [76].

Nanogel is polymerized system with nano size in which polymer is is crosslinked. This comes under the nanotechnology system in which drug is delivering in sustained and controlled way. It is very useful in polymer science technology with nano-size delivery system which proves in clinical trials [82-83]. When we talk about gel then the mind said that it is traditional delivery system but when introduced nano word with gel it's comes under the noval drug delivery system for the specific application. In current reported works described nanogels is very meaningful in gene theraphy and help in cancer treatment [84-85].

The active constituent of mentha piperata is Peppermint which is getting after the extraction of herbal plant. It shows the analgesic effects when it is used topically and also help in headaches [86].

The drug is delivered via topical route; it is most promising route for local and systemic treatment. This is route most preferably because this route provides better absorption of drug and bioavailability [87]. These allopathic drugs, however, are not only cost effective in general, and also have several adverse side effects. Medicinal plants there of re play an ever increasing role for the management to lifelong skin disorder, especially in developing countries where plants are readily available [88].

Skin of human being is very sensitive, we observed rashes, red skin, itchy and sometimes dry skin all these happen due to fungal infection [89]. Fungal infection mainly caused by fungus. For treating such type of fungal infection we all need a localized treatment to drug permeation into the skin so the delivery system is to prepare i.e. topical delivery. This system is selected not only because this have specific site of action but also short biological half time and increase the duration of time [90]. As per reported study, new chemical moiety has been developed, around 40 percent from that shows poor solubility and poor bioavailability to overcome this problem, an alternatively deliver system is developed i.e microemulsion [91]. The microemulsion is prepared with antifungal drug which results minimize the skin irritation increase the permeation of drug and also increasing the drug loading capacity [92-93].

The microemuslion is novel drug delivery system which is thermodynamically stable, ease of preparation, consist of two immiscible liquid (oil/water) stabilized by the surfactant or co-surfactant [94].Traditional formulation have disadvantage over the novel delivery system. The problems arise with the traditional formulation when we use in to treat the fungal infection having low solubilizing capacity with lipophilic drug but in microemulsion formulation improve the solubilising capacity [94].

In recently studies, Nanoemulsion containing eugenol proved reduced induced paw edema in rat which is compared with marketed formulation i.e. gel containing Piroxicam [95]. When we formulate the nanoemulsion formulation having anti-inflammatory drug which enhance the drug efficacy [96]. Another example of nanoemulsion with naproxen prepared at different concentration by using of oil (IPM), surfactact (labrafil-M), and co-surfactant (

Chremophore EL), IPA and sodium hydroxide, this formulation results increase the the rate of permeation, anti analgesic and anti-inflammatory [97].

The use of herbal drug in pharmaceutical products is increase day by day because they have less or no side effects [98]. Already reported in the study that the presence of amygdalin, a glycoside form of benzaldehyde in almond oil showing anti-inflammatory, pain relieving, anti cancer and anti-hepatotoxicity activities [99-100].

Preparation of nanoemulsion formulation with almond oil for the inhibition of inflammation and pain via topical route and literature also confirm the synergistic effects with the combination of NSAID such as ibuprofen that result anti-inflammatory and anti-analgesic properties [101]. Due to high cost and adverse effects of these medicines herbal medicines are in row [102]. Concept of polyherbal formulations are also important because it produces synergy effect due to active compounds present in plants which results to produce better effectiveness [103-104]. A number of herbal extracts used as adjuvants to the management of several diseases without appropriate full actions and constituents. These are assigned to unaffordable cost and adverse effects [105]. Allopathic drugs are available in the market but use of herbal drugs is more effective [106].

The plant Cordia oblique belonging to the family Boraginaceae [107], which have different medicinal value such as analgesic, anti-pyretic, anti-inflammatory. Various compound present in this herbal drug such as flavanoids, alkaloids, phenolic, glycosides and steroid in the methanolic extracts of leaf [108-109].

Herbal drug play a vital role in health services and gaining considerably greater global exposure. Most of the population believes in the use of plant and plant extract for treating our health issue. Herbal drug also have pharmacological activities. The various serious concerns related to the skin such as redness, itching, inflammation which is treated by opoids and anti-inflammatory drugs. There is wide variety of herbal plant which results anti-inflammatory activities, example of some traditional plant are Acacia nilotica, Withania somnifera, Glycyrrhiza glabra, Boswelia serrata, Phyllanthus amarus, Eclipta Alba etc. anti-inflammatory activity from this plant due to presence of flavonoids. The high content of flavanoids presents in Cynodon dactylon, Cassia tora and Cassia alata that results anti-inflammatory activity [110]. These three plants have various therapeutic effects such as anticancer activity, oral anti-inflammatory, anti-bacterial activity, anti-oxidant activity, skin disorder and wound healing activity by plant Cassia tora and Cassia alata and other activity such as anti fungal, anti bacterial, skin disorder and wound healing activity from the plant Cynodon dactylon [110].

TYPES OF FORMULATION/ DOSAGE FORM

Microemulsions are novel drug delivery system which is thermodynamically stable, clear immiscible liquids and surfactants. Microemulsion system improves the solubility & oral bioavailability of poorly soluble drug by enhancing the drug permeability & solubility in gastrointestinal tract. This drug delivery system increases transportation via lymphatic system because of its better permeation rate [111-112].

The stability is due to the presence of large quantities of surfactants. Microemulsion is mainly 3 types [112-113];

1. Oil in water
2. Water in oil
3. Bi-continuous

1. **Oil in water:** In this liquid system, oil is treated with appropriate surfactant which helps the oil to distribute evenly n water
2. **Water in oil:** In this Microemulsion, water should be distributed evenly in oil.
3. **Bicontinuous:** This system involves both water and oil in smilar quantities, exists as continous phase.

METHODOLOGY

1. **Selection and collection of drug**
2. **Morphology of herbal drug**
3. **Identification of drugs**
4. **Extraction of herbal drug**

5. **Solubility**
6. **Selection of oil, surfactant and co-surfactant:**
7. **Preparation of microemulsion**
8. **Identification and Interaction study**

1. **Selection and collection of drug**: First of all selected the herbal drug on the basis of formulation & category of under which the formulation is being used [58].

2. **Morphology of herbal drug**: Drug is identified by morphologically and also examined by under the microscope [58].

3. **Identification of drug**: Identification of herbal drug is carried out by thin layer chromatography and by the instrument FTIR [58].

4. **Identification by Chemical test**: This should be carried by out by qualitative test of herbal drug in this chemically test the chemical moiety is present in the particular herb such as tannins, alkaloids, flavanoids etc [58].

5. Extraction of Herbal plant by Different Method:

For Herbal standardization, herbs are collected and extraction of herb is done by soxhlet apparatus before doing the extraction process first of all ensure that leaf of herb is obtained in powdered form, this powdered placed in soxhlet apparatus for extraction , extraction is done in different solvent on the basis of polarity i.e. Ethyl acetate, Petroleum ether, Hexane , Chloroform, ethanol, methanol and water by doing this we obtained all phyto constituent. After extraction all extracted should be filter separately with help of whatmann filter paper and placed separately and concentrated with the help of rota vapour and placed in dessicator. Calculated the yield of methanolic extract and then for further preparation of gel because it is used in the treatment of pain, edema and fever [109].

Another method for Extraction of herbal drug in this selected herb is dried and powdered with the help of mixer. The powdered drug is pass through the sieve (Sieve no.80) and stored in well closed container so the drug cannot be moisturized by environmental condition. Herbal drug is accurately weigh and transferred into the round bottom flask, placed in heating mantle connected with reflus condenser. This could be done by using 10 percent ethanolic mixture for the period of 4-5 hrs. During this proces , slurry is obtained , that slurry should be filtered by through whatmann filter paper and remaining herbs are again processed with the same mixture for the same period of time.Then again filtered the slurry through filter paper. After that combined both the slurry and make concentrated by using rota vapour. We obtained the thick paste of that, if we want more thick than the this concentrated material placed in water bath in appropriate flask [58]. All the herbal extracts were tested for qualitative and quantitative photochemical, such as total phenol, flavonoid, tannin and saponins contents varied from solvent to solvent. The methanolic extract showed potential therapeutic activities [58].

Extraction of volatile oil from seed (Coriander):

The seed from which oil is extracted first converted into powdered with the help of mixer, with the help of Clevenger apparatus volatile oil is collected in form of oil water mixture by the hydro-distillation method. The obtained oil is extracted with diethyl ether and for making moisture this oil pass through the oil with anhydrous sodium sulphate [65].

Extraction of herbal Plant:

Extraction of herbal plant can also be done by the hot extraction method, in this method whole plant is converted into powdered form than placed for the period of 12 hours in methanol for the extraction, and then we concentrated the extraction into thick paste as desired concentrated [114].
These two plant Cardiospermum halicacbum and Vitex negundo leaves should be extracted by two different methods as per reported in report. First we cleaned the leaved, then dried and afterthat converted the leaves into powdered. Powdered of the herb Cardiospermum halicacbum can be extracted by soxhlet method using methanol as solvent, this should be placed for the period of 72 hours. Powdered for the herb Vitex negundo can be extracted by maceration mehod with the same solvent i.e. methanol for the 7 day. After the completion of period under which both herbs were placed, then filtered the extract and concentrated the extract with the help of rotavapour at particular temperature. The prepared extract is placed at 4 to 8^0C for further use [59].

Solubility:

First of all, we selected the herbal drug from through made a formulation, than we check the solubility of that particular herb in different oils, surfactant and co-surfactant, this is done by dissolving the excess of herbal drug into it. We also selected the oil in combination manner. After that, we examine the concentration of oil surfactant and co-surfactant and oil combination by UV visible spectrophotometer at particular wavelength [115].

Selection of oil, surfactant and co-surfactant:

On the basis solubility criteria we selected the oil, surfactant and co-surfactant for the examination [116].

Microemulsion Preparation

Before doing preparation of formulation first of all identifies the best region in phase diagram. Aqueous phase titration method is most commonly method used for the oil in water microemulsion formulation [117]. After that microemulsion is prepared at desired component ratio. Transparency of the formulation mixtures were observed visually after 48 hours. Only transparent formulations were further analyzed [118-119].

Identification and Interaction study

Identification and Interaction of drug with the excipients can be evaluated with the help of FT-IR. In this Methanolic extract of herb is used to perform the interaction study with prepared gel and compared. The results were obtained the spectrum [120].

PARAMETERS FOR EVALUATION OF MICROEMULSION [117]

Visual observation: Visual observation of the prepared microemulsion physically observed that should be transparent and there is no phase separation. The formulation has no phase separation and shows better transparency goes for further evaluation.

pH: The pH parameter also evaluated of the prepared microemulsion by the calibrated pH meter. It is done by placing the electrode in the formulation and wait for the minute to get the result.
pH is calibrated by the standard solution having range 4.9 to 7.9. Standard range of the prepared formulation is 4.5 to 7.5, this means formulation neither be basic nor be acidic.

Density: Pycnometer is used to determined the density of the formulation i.e. oil-in-water microemulsion. First of all, weigh the empty pycnometer via electronic balance and then fill the water in it and then re-weigh the pycnometer. After substracting the empty pycnometer weight from the total weight of filled water by this we get the weight of water via this we get density of water. After that formulation fill the pycnometer and weigh on the electronic balance and note down the weight, this confirm the volume of formulation.
Formula:

Density (g/mL): weight (g)/Volume (mL)

Electrical Conductivity: Electrical conductivity test is carried out of the sample only after the calibration of conductivity meter by distilled water, so we get the accurate result from that instrument. The electrical conductivity of the formulations was determined by Nano partica SZ 100. The electric conductivity was calculated in mS/cm.

Viscosity: The viscosity of the prepared micro emulsions were measured by using Nano Partica SZ 100. Those formulations have less viscosity which is better for the administration; this formulation is also selected for further evaluation.

Size analysis of Microemulsion droplet: Size analysis of droplet is done at fixed refractive index of tested formulation and is determined by photon spectroscopy which works on Brownian motio of the particles [117].

Tranmission emission microscope:
TEM instrument is used to check the morphology and structure of the formulation i.e. microemulsion [28].

Emulsion stability test: Change in droplet size over the period of 24 hours, 7 days and 28 days were analyzed to determine kinetic stability of the microemulsion [121-122]. The microemulsion was centrifuged at 3000 RPM (Eppendorf Centrifuge 5430 R) for half an hour.

Zeta potential analysis: The surface charge of the microemulsions are measured by the SZ-100 by measuring the zeta potential of a solution. The sample is injected into a cuvette and placed in the particle size analyzer and the zeta potential of the prepared microemulsion was measured. The zeta potential is mostly used to check of dispersion stability of a sample [123].

PARAMETERS FOR EVALUATION OF GEL

1. Organoleptic
2. Viscosity
3. Extrudability
4. Spreadability
5. pH
6. Homogeneity
7. Grittiness
8. Stability Study
9. Skin Irritation Test

1. **Organoleptic test**: In this physical appearance, color, texture etc. were observed [9].

2. **Viscosity:** Brookfield viscometer instrument is used to determine the viscosity of gel formulation. Generally the Spindle No.7 is used for determination of viscosity of gel [9]**.**

3. **Extrudability:** The gel having the Extrudability property and classified into three categories in which result shows more than 90 percent than the gel comes in excellent category, if result is more than 80 but less than 90 its comes under good category and last is greater than 70 percent but not more than 80 percent shows fair. In this experiment, 500 g of sample is filled in standard capped collapsible tubes & is sealed. Then the filled sample is placed over the two clamped glass slides [124].

4. **Spreadability**: Spreadilbilty is done by using speadibility apparatus consist of wooden block and a pulley at

one end. Slip and drag property of tested gel formulation is accurately measured by this method [125]. Spreadilbilty is calculated as:

$$S = (MxL)/T$$

Where,

S- Spreadability

M- Weight in the pan (Tied to the upper slide)

L- Length moved by the glass slide

T- Time (in sec.) taken to separate the upper slide from the ground slide.

5. **pH:** The pH of the formulation is evaluated by preparing 1% and kept for two hrs, then note the reading flashed in the pH meter and record the result. All the sample reading should be taken in triplicate after that average value should be considered for the final result. This evaluation should be done on the calibrated pH meter [126-128].

6. **Homogeneity:** Homogenity test is carried out by visual inspection, appearance and presence of aggregates. This should be carried out on the finished formulation [126-128].

7. **Grittiness:** Grittiness test should be done to evaluate particulate matter under light microscope in any desired gel formulations [126-128].
8. **Stability study:** Stability study follows ICH guidelines. This study is carried out on any gel formulations at different temperature and humidity i.e. real time condition, room temperature and accelerated condition. This test should be done for three months. The Evaluating parameters during this period are appearance, pH and Spreadability [129-130].
9. **Skin irritation test:** Skin irritation test of gel formulation is done on model animal i.e. wister rate with average weight of 150-200 g. Three days before the test, hair should be removed from the rat. In this study erythema and edema on treated skin should be observed on daily basis for 7 days [131].

PARAMETERS FOR EVALUATION OF OTHER TOPICAL FORMULATION

Solubility:

First of all, we selected the herbal drug from through made a formulation, than we check the solubility of that particular herb in different oils, surfactant and co-surfactant, this is done by dissolving the excess of herbal drug into the fixed quantity of oil, surfactant and co-surfactant. We also selected the oil in combination manner. After that, we examine the concentration of oil surfactant and co-surfactant and oil combination by UV visible spectrophotometer at particular wavelength [28].

Estimation of drug content:

For determining the active constituent from the gel formulation, the instrument is used to find the drug content in the formulation UV Visible spectrophotometer, read the reading at particular wavelength. Sample and standard is prepared as specific concentration by using methanol as a diluent, this diluents use as blank when take reading. Precaution should take when we prepared the sample; sample should be filtered through whatmann filter paper before take the reading in spectrophotometer [132].

Centrifugation test:

Prepared microemulsion undergoes under the test of centrifugation. It is done by filled the formulation in centrifuge tube and place on the centrifuge instrument, after that we set the RPM and time in the instrument before starting the instrument. It is all done at room temperature. When the cycle is completed, sample is withdrawn from that than go for physical observation i.e.no change in formation [28].

Acute Toxicity Testing:

Test is performed to know pharmacologically activity on animal (mice), first of all we do the acute toxicity test in this drug is induce as a single dose to each animal for the estimation gross behavior & LD50. LD50 defined as the dose which kills the animal 50 percent in last 24 hrs [58].

Primary dermal Irritation Index: This test carried out for acute reactions when the formulation is applied to topically to the skin The PDII give the information regarding skin i.e. skin is irritating or non-irritating [51].

Animal selection: Before doing the animal study, we all need clearance or approval from the committee for the purpose of control and supervision of experiments on animal (CPCSEA) which constituted by the central government. For this study we required healthy animals. The entire animals placed in space where the facilities are available for the animals such as drinking water, feeding material and enough space for the movement with a sufficient light in a room [51].

Preparation of animals:

For performing this study, the selected animals should have intact skin and healthy. Animal fur is removed from the skin one day before for the experiment [51].

Application of the herbal gel to the skin of animal:

Formulation is applied in the form of gauze patch to the skin of animal which is held loosely to the skin, this should be removed after the period of 4 hrs, and then we observed the skin of animal. Same procedure is to be done with the formulation don't have active herb component on it. Again we observed the skin of the animal after the period of one hrs i.e this is considered as control animal. Every day we observed the control and test animal to check the skin irritation or toxic reaction i.e. oedema or erythema. We rating the reaction 0 to 4, this means zero rating considered no skin erythema or oedema; one means very slight; two means defined; three means moderate and four means severe oedema or erythema [30]. It is calculated as;

$$\text{Primary Dermal Irritation Index (PDII)} = \frac{\text{PDII observed on } 12 + 24 + 48 + 72 \text{ hrs}}{4}$$

Classification of system as per PDII [51]:

PDII value	Condition
Less than 0.5	Non -irritaing
0.5 to 2.0	Slightly irritaing
2.1 to 5.0	Moderately irritating
More than 5.0	Severely irritating

Hematological analysis:

After the 14th and 28th of the test performed to the animals, their blood sample collected by vein puncture and test of haemoglobin is performed by instrument haemocytometer [51].

In vitro diffusion test:

There is two compartments in Franz diffusion cell, first is receptor compartment and second one is donor compartment. Phosphate buffer is placed in the receptor compartment which freshly prepared and the sample is put in donor compartment. Instrument is started by setting a fixed RPM and temperature, the sample are withdrawn at time to time at fixed interval and after that buffer is placed against the sample withdrawal quantity. The formulated sample was determined by spectrophotometer at particular wavelength, most of the time use methanol as blank because sample is prepared in methanol as solvent. Many kinetics equations were performed after completion of in vitro diffusion study [114].

Drug release kinetics:

To perform the release kinetics of drug in different kinetic model by obtained the data from in vitro release of formulation [133].

In Vitro antifungal activity:

In vitro antifungal activity is carried out with the help of Franz diffusion cell via diffusion menthod [134]. The Various strains were used for performing the in vitro test such as Aspergillus fumigates (MTCC 6500), Aspergillus niger (MTCC 282) and Candida albicans (MTCC 4748) and also used some media for this test like Czapek Dpx media having the pH value 6.8 and Sabouraud Dextrose agar wirh pH 6.2. We prepared the media with the help of distilled water after that media is autoclaved for 20 minutes at 121^0C. the prepared media transferred under the laminar flow into the petridish which is previously sterile along with mixed strain. Different dilution is prepared as different concentrations and known concentration of sample incubated as per condition which is require for incubation for a certain period of time [76].

Histopathological Investgations:

First we sacrifice the animal through Cervical Dislocation, than go for further studies. The organ such as spleen, thymus, bone joints of ankle joint were separated out and also removing the excessive superficial fat which is deposited to skin of animal and then weigh. The Cal-Ex Decalcifying solution is used for the isolation of ankle joint; the isolation is done for 9 days. After the completion of 9 days isolated ankle joint is treated with paraffin, then we go for section cutting this mean we prepared the slide using Harris hemotoxylin and Eosin [135] as a strain then we observed the histopathological changes in prepared slide with the help of digital Microscope so we recorded the results.

Optimization of formulation (32 full factorial Design):

This factorial design is used after knowing the concentration of range of the formaulation such as oil range (2.5 to 10 percent w/w), and Smix (50 to 60 percent w/w) of trial batches [136]. The prepared formulation is evaluated and obtained value is compared with value obtained mathematical model [94].

Skin irritation test:

When the prepared formulation is applied to affected area of the skin, the irritation test is very important. So before doing topical application, it is necessary to perform. Skin irritation test of gel formulation is done on model animal i.e. Rabbit. In this experiment, the depilation of back of rabbit and then applied the the formulation to two different animal model i.e. rabbit, gauze and adhesive bandage was used covered that particular treated area and this should be removed after one day. In this study we graded the skin of animal model such edema and erythema. Scoring could do after 72 hours post. Score tells the formulation is non-irritant, irritant and highly-irritant [137-138].

The total scores for irritation test were calculated using the following equation:

$$\text{Average irritation scores} = \frac{(\text{Erythema reaction scores} + \text{Edema reaction scores})}{\text{Time interval } (h)}$$

In-vitro antifungal activity (cup plate method) [139]

Test is performed by using cup plate method, in this vertical cylinder is used for diffusing the antifungal through solidify agar in a petri-plate where we prevent the growth of micro-organism [140]. Candida albicans culture should be grown after placing overnight of it then we inoculated into the sterilized media. The formulation is selected for further testing is carried out [140].

Problems:

The drugs which are very poorly soluble in GI fluid, that drug have low bioavailability. We find that around 50% of marketed drug have low oral bioavailability. Some drug delivery system has developed to improve the oral bioavailability of drug. Allopathic medicines those used for treatment of diseases not only of high cost and possess so many adverse effects [141]. Hence, it is important to explore drugs from traditional medicinal plants those will hold effective results in handling of Skin diseases with no adverse effects [142-143].

Plant based therapy is becoming more important [144].There is a increasing need to develop new drugs for skin disorder from plant sources as result of the high cost and side effects of current drug therapies [145]. Allopathic medicines those used for treatment of skin diseases not only of high cost and possess so many adverse effects [146]. The therapeutic effectiveness of most medicinal plants used to treat skin diseases such as inflammation. Hence, it is important to explore drugs from traditional medicinal plants those will hold effective results in handling of skin disease with no adverse effects [147-148].

Nows a days, a increasing need to develop new drugs for skin disorder from plant sources we observed the non-steroidal anti-inflammatory drug, immunosuppressant drug, anti-rheumatic, those drugs produce many of side effects such as gastrointestinal and hormonal disturbances. Today scenario , we all approach the drug which have less or no side effects [59].

Skin disorder/Diseases are serious concern. Many times skin diseases occur due to fungal or some time pain in joint, this is happen with the age factor because joint pain problem mainly happen in old age person. There are so many oral drugs is advise for the treatment of skin diseases but some serious concern arise when we use such type of drug because these drug result serious adverse effect also increase the cost of treatment. So, instead of using allopathic drug today we us herbal drug for the treatment of such type of diseases which decreases the cost of treatment and minimize or no side effect.

A number of herbal plants effective in anti-inflammatory activity. Researchers is always tried to provide the effective treatment to the patient who is affected to a particular disease [149-150].

Solutions:

Mostly, in case of skin diseases cosmetic formulation are used because medicine comes in direct contact with the skin and facilitate instant relief. Thus, for cosmetic formulation Microemulsion delivery system proved an advantage because it has high permeability, improves absorption of lipophilic drug and also showed low skin irritation. Microemulsion formulation is selected on the basis of transparency which can be visually observed. Transparency is increased with increase the concentration of surfactant than those formulation gives for further analysis. There should

be no phase separation after test by centrifugated at 3000 rpm. Zeta potential parameter confirms that droplets of those formulations were stable [151].

Conventionally used herbal medicines are gaining significantly greater worldwide exposure. Public funding for worldwide traditional medicinal herbal research is also growing. WHO is also inspiring the safe and effective use of herbal medicines. Since many years herbal medicines are a dependable, satisfactory and desirable treatment option for people around the world. Market value of herbal medicines is mounting day by day and the number of investors in the production and research of herbal medicines is growing because of the same reason. Allopathic medicinal products present significant adverse effects but allopathic medicinal products are of very high quality. Only solution was found the use of herbal drugs in the treatment of skin diseases without any side effects, and these herbal drugs are also comparatively cheaper.

Antibiotic category of drug is used to treatment of acne diseases. When we talk about the treatment of acne disease we consider that 2-3 months is required when the the drug is applied topical [152-153]. When the same treatment applied for the longer period, also increase the cost of treatment and also increase chance of side effect and also observed the the human skin resistance [154] to particular bacteria that's why we adopt the natural therapy to treat such type of disease, which reduced the cost of treatment and also minimize or no side effect and there is chance of no bacterial resistance. The herbal drug was used as natural therapy to treat the topical infected disease and also developed the formulation with the use herb for future prospective [65]. Coriandrum sativum drug have also used as anti-inflammatory [155] and anti microbial properties which effectiveness against bacteria and fungi [156].

In the pharmaceutical, there are several dosage forms available such as ointment, creams and gels etc. From this formulation, Gels is most effective formulation. In this study we formulate the herbal gel by using the methanolic extract of Cordia oblique leaf which is used in the treatment of inflammation, pain and fever, also easy to apply and no skin irritation due to this there is less chance of side effects [109].

Traditionally used herbal medicines are gaining considerably greater global exposure. The trend of herbal research is accelerated internationally for the research project by this we get the fund.. WHO is also encouraging the herbal drug for their effectiveness. From last many years herbal drug are a safe , reliable and satisfactory for people around the world. Day by day marketed value of herbal drug is increased [157] and the number of researchers works on herbal drug for the same reason. Allopathic drug raise the major concern i.e. Side effects or adverse effects.

Conclusion:

In India, we find that today scenario demand the specific drug delivery to the specific site of action and competent way. Microemulsion, a novel approaches to the drug delivery system because this formulation is physically stable, good in appearance, homogeneity, extrudability, and spreadability. In this drug delivery we use natural oil in the formulation which are pharmacologically active, this is very important for further study. So we introduced that formulation for commercial use to the future.

The study evaluate that the transdermal system of herbal drugs describe that we increase the permeation of drug via this route [28]. In now days we observe that the researcher is very keen to remodel the delivery system for the permeation of drug via skin through topical system [158]. When we discussed the previous study of the curcumin as active constituent in the formulation, not only for the enhancement of drug permeation, but also the gel matrix formulation by using this chemical constituents for increasing the bioavailability and stability of the formulation [30].Curcumin is active constituent of the herbal drug comes in the category of anti-inflammation which help in reducing the inflammation. This delivery system is used for providing better therapeutic effect, so nanoemulsion formulation containing curcumin is most advancement technique for transdermal system [30].

The various chemical constituent were present in Cardiospermum halicacbum and Vitex negundo. Out of various chemical constituent luteolin and apigenin is most active chemical moiety which shows the anti-arthritic activity. So, when we use the methanolic extract of these two chemical constituent and prepared herbal formulation for topical delivery for the treatment of arthristis [159].

The dermatological fungal infection is treated with high molecular drug to reduce the nephrotoxicity with less solubility which is clinically proved for systematic effectiveness. In this study we prepared a nanoscale nanomulsion system with carrying a antifungal drug for the topical route to increase the skin permaeation. This formulated system is safe and more effective for cutanious infection as comparison to oral route. So, we conclude that this formulation is more suitable for the treatment of fungal infection and also economically approved. According to the study, we find that Nanoemulsion containing Amphotericin B (AmB) is currently unavailable in the market [76]. Even a single formulation of AmB in nanoemulsion (NE) form is not available in the market for the topical application.
The formulation containing coriander oil formulated for the treatment of acne diseases, which is commercialized in past decade on human being.

Reported study concluded that the microemulsion formulation increases the drug permeation and solubility of drug, the prepared formulation retain maximum time at the site of action. The formulation result that there is no change in physical, also exhibit stability when be placed at room temperature to specific period of time.

Methanolic extract of the plant such as Gymnema and Andrographis is natural drug have potential to treat the skin diseases like inflammation. This may be help to reduce the inflammation because of presence of β-sitosterol.
In this study, we conclude that the methanolic extract of Cordia oblique leaf is used as a anti-inflammatory activity, this extract also used to treat the inflammation, pain and pyrexia [109].
Study concluded that almond oil containing nanoemulsion with or without ibuprofen for topical delivery system prepared that results enhance analgesic and anti inflammatory effects [101].
Studied reported that the polyherbal prepared by methanolic extract of the Cynodon dactylon, Cassia tora and Cassia alata which gives anti-inflammatory activity due to the presence of flavanoids and steroids. The formulation provides the synergetic effects when formulation prepared with methanolic extract of herbs along with diclofenac sodium, that formulation very useful for the treatment of inflammation [109].

Scope for further work:

Present studies shows that herbal drug are more effective in handling of skin diseases such inflammation , acne and fungal infection in comparison to allopathic medicines because of herbal drugs have no side effects and cost effectiveness. These herbal drugs show good and effective results to treat skin diseases such as anti-inflammatory. In future we have to recognize different active constituents present in these anti-inflammatory drugs of different chemical natures. We can further precede this anti-inflammatory activity to other plant extract comprising the same basic moiety on the basis of Structural relationship activity. We can find some other activity as in this above study flavonoids is accountable for antioxidant activity. We can make combined formulation using more than one herb. Future aspect of present study is to grow the novel drug delivery system.

Novel drug delivery system is important to eliminate the several factors like low bioavailability, in vivo stability, gastrointestinal absorption and one more unspecific site of action of herbal drugs [160]. Future aspect of present study is to grow the novel drug delivery system.
When we talk about the future prospective, then we go clinical trial for the topical herbal based formulation prepared by using the Cardiospermum halicacbum and Vitex negundo as herbal drug, in which luteolin and apigenin is active chemical constituent for the patient who suffer from joint disorder. This herbal based formulation proved significant role in future prospect [59].

Many of herbal drug were used for the treatment of inflammation, arthritis, analgesic, skin disorder. We also work on it to formulate different preparation for skin disorder patients [161].

Now days, traditional herbs are used in skin diseases. For the future point of view we have to collect all the traditional herbs which results anti-inflammatory, anti-arthritis, anti-pyretic, ant-analgesic etc these herbs are beneficial to the society also we have the duty to identify the herbal drug which shows the anti-inflammatory action, so work on that herbs and find out the another active moiety from the that particular herb for the treatment of such type of diseases.

Another aspect of present study is to develop the novel drug delivery system. It is very important to identify the active compound present in herbal plant which is used to treat the particular disease. We can make combined formulation using more than one herb.

References:

1. Nora A. AlFarisa, Ghedeir M. Alshammarib, Muneer M. Alsayadic, Munirah A. AlFarisd,Mohammed A. Yahyaba , "Antidiabetic and antihyperlipidemic effect of Duvalia corderoyiin rats with streptozotocin-induced diabetes" Saudi Journal of Biological Sciences; Volume 27, Issue 3, March 2020, Pages 925-934
2. Rang HP, Dale MM, Ritter JM, Flower RJ. Anti-inflammatory and immunosuppressant drugs. In: Rang and Dale's Pharmacology. 6th ed., Ch. 14. Edinburgh: Elsevier Publications; 2008. p. 226-45.
3. Prentis RA, Lis Y and Walker SR: Pharmaceutical innovation by the seven UK-owned pharmaceutical company. Br J Clin Pharmacol 1988; 25: 387-96.
4. Lipinski CA: Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 2000; 44: 235-49.
5. Leuner C and Dressman J: Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm 2000; 50: 47-60.
6. Chivate, N., Chivate, A., Wadkar, K., Shah. R., (2020) Design and characterization of self emulsifying drug delivery system of herbal drug compound, International Journal of Pharmaceutical Sciences and Research 11 (6), 2761-2766.
7. Humberstone AJ and Charman WN: Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev 1997; 25(1): 103-28.
8. Porter CJ and Charman WN: Intestinal lymphatic drug transport: An update. Adv Drug Deliv Rev 2001; 50(1-2): 61-80.
9. Giri, A.M., Bhalke, D.R., (2019) Formulation and evaluation of topical anti-inflammatory herbal gel, Asian Journal of Pharmaceutical and Clinical Research 12(7), 252-255.
10. Nadkarni KM, editor. Indian Meteria Medica. Bombay, India: The Karnataka Printing Press and the Popular Press Ltd.; 1976. p. 480-4.
11. Kirtikar KR, Basu BD. Indian Medicinal Plants. Allahabad: Basu Ltd.; 1935. p. 488
12. Rehman H, Yasin KA, Choudhary MA, Khaliq N, Rahman A, Choudhary MI, et al. Studies on the chemical constituents of Phyllanthus emblica. Nat Prod Res 2007;21:775-81.
13. Charaka SC. Kavyamala Series of Books. Bombay, India: Nirnaya Sagar Press; 1941. p. 114-5.
14. Liu X, Cui C, Zhao M, Wang J, Luo W, Yang B, et al. Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities. Food Chem 2008;109:909-15.
15. Thilakchand KR, Mathai RT, Simon P, Ravi RT, Baliga-Rao MP, Baliga MS. Hepatoprotective properties of the Indian gooseberry (Emblica officinalis gaertn): A review. Food Funct 2013;4:1431-41.
16. Baliga MS, Dsouza JJ. Amla (Emblica officinalis gaertn), a wonder berry in the treatment and prevention of cancer. Eur J Cancer Prev 2011;20:225-39.
17. Baliga MS, Meera S, Mathai B, Rai MP, Pawar V, Palatty PL. Scientific validation of the ethnomedicinal properties of the ayurvedic drug triphala: A review. Chin J Integr Med 2012;18:946-54.
18. Kumar A, Singh A, Dora J. Essential perspectives for Emblica offcinalis. Int J Pharma Chem Sci 2012;1:11-8.
19. Khan KH. Roles of Emblica officinalis in medicine – A review. Bot Res Int 2009;2:218-28.
20. Zhang LZ, Zhao WH, Guo YJ, Tu GZ, Lin S, Xin LG. Studies on chemical constituents in fruits of tibetan medicine Phyllanthus emblica. Zhongguo Zhong Yao Za Zhi 2003;28:940- 3.
21. Rehman H, Yasin KA, Choudhary MA, Khaliq N, Rahman A, Choudhary MI, et al. Studies on the chemical constituents of Phyllanthus emblica. Nat Prod Res 2007;21:775-81.
22. Saravanan G., Ponmurugan P. "Ameliorative potential of S-allyl cysteine on oxidative stress in STZ induced diabetic rats", Chem Biol Interact, (2011)189, 100–106: 591.
23. G. Ulrich-Merzenich, D. Panek, H. Zeitler, H. Vetter, and H. wagner, "Drug development from natural products: exploiting synergistic effects", Indian Journal of Experimental Biology.
24. E. M. Williamson, "Synergy and other interactions in phytomedicines". Phytomedicine, (2001), vol. 8, no. 5, pp: 401–409.
25. World Health Organization. "Diabetes. Fact sheet N°312. World Health 324 Organization" (2011):10.1017/CBO9781107415324.004
26. J. Wall, "Antioxidants in prevention of reperfusion damage vascular endothelium". The Trinity Student Medical Journal,(2000), vol.1: pp. 67–71.
27. D'Amico, D.Davidovic-Amic,D. Beslo, and N. Trinajstic, "Structure radical scavenging activity relationships of flavonoids," Croatia Chemica Acta., (2003), vol. 76, pp: 55–61.
28. Jignesh D. Modi, Jayvadan K. Patel, Nanoemulsion-Based Gel Formulation of Aceclofenac for Topical Delivery. International Journal of Pharmacy and Pharmaceutical Science Research 2011, 1 (1) 6-12
29. Attwood D, Mallon C, Ktistis G, Taylor CJ. A study on factors influencing the droplet size in non-ionic oil-in-water microemulsions. Int J Pharm. 1992; 88:417Y422.
30. Rachmawati et al., Curcumin Nanoemulsion for Transdermal Application:Formulation and Evaluation Research and Development on Nanotechnology in Indonesia, Vol.1, No.1, 2014, pp. 5-8 RDNI, Vol.1, No.1, 2014, pp. 5-8
31. Sharma S, Parwa S, Jain UK. Development and evaluation of topical gel of curcumin from different combination of polymers formulation & evaluation of herbal gel. Int J Pharm Pharm Sci. 2012;4:452-456.
32. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm. 1995; 114: 103Y110.

33. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez-Quintela MJ, Marty MA, Guy JP. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur J Pharm Biopharm. 1997; 43:37Y42.
34. Dreher F, Walde P, Walter P, Wehrli E. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J Control Rel. 1997; 45:131Y140.
35. Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC, eds. Encyclopedia of Pharmaceutical Technology. vol. 9. New York, NY: Marcel Dekker; 1995:375Y421.
36. Escribano E, Calpena AC, Queralt J, Obach R, Domenech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci. 2003; 19:203Y210.
37. Faiyaz S, Shafiq S, Sushma T, Farhan J, Khar R, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation, Eur. J. Pharm. Biopharm. 2007; 66: 227– 243.
38. Chen H, Chang X, Du D, Li J, Xu H, Yang X, Nanoemulsion based- hydrogel formulation of ibuprofen for topical delivery, International Journal of Pharmaceutics 315(1-2):52-58.
39. Gasco MR, Gallarate M, Pattarino F. In vitro permeation of azelaic acid from viscosized microemulsions. Int J Pharm. 1991; 69:193Y196.
40. Gonzalez E, Cruz C, Nicolas R, Egido J, Herrero-Beaumont G. Long-term effects of nonsteroidal anti-inflammatory drugs on the production of cytokines and other inflammatorymediators by blood cells of patients with osteoarthritis. Agents Actions. 1994; 41:171Y178.
41. Gosh MN. Fundamentals of Experimental Pharmacology. Kolkata, India: Hilton and Company; 2005:192
42. Kawakami K, Yoshikawa T, Moroto Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs, I: prescription design. J Control Rel. 2002; 81:65Y74.
43. Kazimiera A, Katarzyna Z, Agnieszka H, Adam J. Biocompatible nanoemulsions of dicephalic aldonamide-type surfactants: Formulation, structure and temperature influence. Journal of Colloid and Interface Science 2009; 334: 87–95.
44. Kemken J, Ziegler A, Muller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm Res. 1992; 9:554Y558.
45. Kreilgaard M, Kemme MJB, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res. 2001; 18:593Y599.
46. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterization and transdermal drug delivery potentials of microemulsion systems. J Control Rel. 2000; 69:421Y433.
47. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by in-vitro microdialysis. Pharm Res. 2001; 18: 367Y373.
48. Bhattarai N, Shrestha G. Antibacterial and Antifungal Effect of Eupatorium adenophorum Spreng against Bacterial and Fungal Isolates. Nepal Journal of Science and Technology 2009; 10:91-99.
49. Negi A, Semwal A. Antimicrobial potential of Eupatorium adenophorum Spreng. Pharmacognosy Journal 2011; 2(18):61-64.
50. Mandal SK, Boominathan R, Parimaladevi B, Dewanjee S, Mandal SC. Analgesic activity of methanol extracts of Eupatorium adenophorum Spreng leaves. Indian J Exp Biol 2005; 43(7):662-3.
51. Negi A, Sharma N, Siingh Mamta F. Formualtion and evaluation of an herbal anti –inflammatory gel containing Eupatorium Leaves Extract. Journal of Pharmacognosyand phytochemistry 2012; 1(4):112-117.
52. Chakravarty AK, Mazumder T, and Chatterjee SN. Anti-Inflammatory Potential of Ethanolic Leaf Extract of Eupatorium adenophorum Spreng Through Alteration in Production of TNF-α, ROS and Expression of Certain Genes. Evidence-Based Complementary and Alternative Medicine 2011; Article ID 471074, 10 pages doi:10.1093/ecam/neq033
53. Gilbert, M.G. & Phillips, S.M., 2000. A review of the oppositeleaved species of Portulaca in Africa and Arabia. Kew Bulletin, 55:769–802.
54. Gerhardt, Philipp, R. G. E. Murray, Willis A, Wood, Noel R. Krieg. 1994. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D. C. (p. 619, 642, 647).
55. N. A. Patel et al / Intl. R. J. of Pharmaceuticals, 2011; 01(01): 15-20.
56. Remington JP. Remington: The science and practice of pharmacy, 21st edition, Lippincott Williams & Wilkins, 773-774.
57. H.A.Sawarkar, S.S.Khadabadi, D.M.Mankar, I.A.Farooqui, N.S.Jagtap. Development andBiological Evaluation of Herbal Anti-Acne Gel. Int.J. PharmTech Res.2010;2(3): 2028- 2031.
58. Syed Umar Farooq et al. Formulation and Evaluation of Novel Herbal Gel of Portulaca Quadrifida for the Treatment of Acne, Indo American J of Pharm Sci 2015:2(4):843-850.
59. R. Aiyalu, A. Govindarjan, A. Ramasamy, Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences (2016) vol. 52, n. 3, jul./sep., 2016,493-506
60. Nair, C.K.N.; Mohenan, N. Medicinal plants in India with special reference to Ayurveda. Delhi, India: NAG Publisher, 1998.
61. Tamhankar, C.P.; Saraf, M.N.; Anti-arthritic activity of Vitex negundo Linn. Indian J. Pharm. Sci., v.56, n.1, p.158-159, 1994.
62. Telang, R.S.; Chatterjee, S.; Varshneya, C. Studies on analgesic and anti-inflammatory activities of Vitex negundo Linn. Indian J. Pharmacol., v.31, p.363-366, 1999
63. Ghosh, M.N. Fundamentals of experimental pharmacology. Kolkatta: Scientific Book Agency, 1984. p.156-157
64. Kumar, E.; Mastan, S.K.; Amrander Reddy, G.; Ragunandan, N.; Sreekanth, N.; Chaitanya, G. Anti-arthritic property of the ethanolic leaf extracts of Cardiospermum halicacabum Linn. Biomed. Pharmacol. J., v.1, p.2, 2008.
65. Aditi Vats1*, Pranav Sharma1 Formulation and evaluation of topical anti acne formulation of coriander oil International Journal of Pharmacy and Pharmaceutical Science Research 2012; 2(3): 61-66.
66. Verma Sheetal, Singh SP. Current and Future Status of herbalmedicines.VeterinaryWorld.1(111)(2011)347- 350.
67. Stan-posthumud JJ, Vink J, Bruijn JA. Topical tretionin under occlusion on a typical navei. 548 (1998)120-130
68. Goulden Victoria. Guidelines for the management of acne vulgaris in adolescents. Pediatr. Drugs. 5(2003): 301-313

69. Kurokawa Ichiro, Danby William .W et al. New developments in our understanding of acne pathogenesis and treatment. Exp. Dermatol.18 (2009) 821- 832.
70. Bowe P Whitney, Logan C Alan. Clinical implication of lipid peroxidation in acne vulgaris: old wine in new bottles. Lipids in Health and Disease 9(2010)140
71. Leyden J. James. The evolving role of Propionibacterium acnes in acne. Seminar Cutaneous Medicine Surgery3 (2001) 139-143.
72. Melo A De Enayde, Filho M Jorge. Characterisation of antioxidant compounds in aqueous coriander extract. Lebensm Wiss u Technol.38(2005)15-19.
73. Pathan IB, Setty CM. Chemical penetration enhancers for transdermal drug delivery systems. Trop J Pharm Res. 8(2009): 173-179.
74. Kleinberg M. (2006). What is the current and future status of conventional amphotericin B. Int J Antimicrob Agents 27S: S12–16.
75. Nahar M, Mishra D, Dubey V, et al. (2008). Development, characterization and toxicity evaluation of amphotericin B-loaded gelatin nanoparticles. Nanomedicine NBM 4:252–61.
76. Afzal Hussain, Abdus Samad, S. K. Singh, M. N. Ahsan, M. W. Haque, A. Faruk & F. J. Ahmed (2016) Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation, Drug Delivery, 23:2, 642-657,)
77. Sonneville-Aubrun O, Simonnet JT, L'Alloret F. (2004). Nanoemulsions: a new vehicle for skincare products. Adv Colloid Interface Sci 108–9: 145–9.
78. Tadros T, Izquierdo P, Esquena J, et al. (2004). Formation and stability of nanoemulsions. Adv Colloid Interface Sci 108–9:303–18.
79. Solans C, Izquierdo P, Nolla J, et al. (2005). Nanoemulsions. Curr Opin Colloid Interface Sci 10:102–10.
80. Formariz TP, Sarmento VHV, Silva-Junior AA, et al. (2006). Doxorubicin biocompatible O/W microemulsion stabilized by mixed surfactant containing soya phosphatidylcholine. Colloids Surf B Biointerfaces 51:54–61.
81. Formariz TP, Urban MCC, Silva-Ju´nior AA, et al. (2005). Microemulso˜es e fases li´quidas cristalinas como sistemas de liberac¸a˜de fa´rmacos. Braz J Pharm Sci 41:301–13.
82. Phillips MA, Gran ML, Peppas NA. Targeted nanodelivery of drugs and diagnostics. Nano Today.2010, 5,143-59.
83. Malmsten M, Bysell H, Hansson P. Biomacromolecules in macrogels – Opportunities and challenges for drug delivery. Curr.Opin. Colloid IN. 2010, 1, 6-18.
84. Hasegawa U, Nomura ICM, Kaul SC, Hirano T, Akiyoshi K. Nanogel quantum dots hybrid nanoparticles for live cell imaging. Biochem. Biophys Res. Commun. 2005,331,917-921.
85. Wu W, Aiello M, Zhou T, Bernila A, Banerjee P, Zhou S. In situ immobilization of quantum dots in polysaccharide based nanogel for integration of optical pH sensing, tumour cell sensing and drug delivery. Biomaterials. 2010,31,3023-31.
86. Gong Y, Fan M, Gao F, Hong J, Liu S, Luo S, Yu J, Huang J. Preparation and characterization of amino functionalized magnetic nanogels via photo polymerisation for MRI applications. Colloids Surf, B. 2009, 71,243-47.
87. Kaur LP, Guleri TK. Topical Gel: A recent approach for novel drug delivery. Asian J Biomed Pharm Sci. 2013;3(17):1-5.
88. Ulrich G. - Merzenich, Panek D., Zeitler H.,Vetter H, and wagner H., "Drug development from natural products: exploiting synergistic effects", Indian Journal of Experimental Biology
89. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh, UK: Churchill Livingstone; 2005. p. 666.
90. Mitchel J. Transdermal drug delivery. Americal academy of dermatology– 58th Annual Meeting, San Francisco, CA, 2000.
91. Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of clopidogrel. Iran J Pharm Res 2010;9:327-34.
92. Yamane M, Williams A. Effect of terpenes and oleic acid as skin penetration enhancers. Int J Pharm 1995;116:237-51.
93. Touitou E, Godin B, Karl Y, Bujanover S, Becker Y. Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J Control Release 2002;80:1-7.
94. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. Int J Pharma Investig 2012;2:140-9.
95. Esmaeili, F., et al., Anti-inflammatory effects of eugenol nanoemulsion as a topical delivery system. Pharmaceutical Development and Technology, 2015: p. 1-7.
96. Eid, A.M., et al., Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. Journal of Nanomedicine & Nanotechnology, 2014.
97. Okur, N.Ü., et al., Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. International journal of pharmaceutics, 2011. 416(1): p. 136-144.
98. Calixto, J., Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research, 2000. 33(2): p. 179-189.
99. Yang, H.-Y., et al., Amygdalin suppresses lipopolysaccharide-induced expressions of cyclooxygenase-2 and inducible nitric oxide synthase in mouse BV2 microglial cells. Neurological research, 2013.
100. Wang, L., et al., Design and optimization of a new self-nanoemulsifying drug delivery system. Journal of colloid and interface science, 2009. 330(2): p. 443-448.
101. Mosayeb Azizi, Fariba Esmaeili, Ali Reza Partoazar, Shahram Ejtemaei Mehr & Amir Amani (2017): Efficacy of Nano- and Microemulsion-Based Topical Gels in Delivery of Ibuprofen: an In-vivo study, Journal of Microencapsulation, DOI: 10.1080/02652048.2017.1316324
102. Patel, S. S. 2016. "Cerebrovascular complications of diabetes: alpha glucosidase inhibitor as potential therapy," Hormone and Metabolic Research, vol. 48, no. 2, pp. 83–91,
103. Dzeufiet P. D. Djomeni, F. Ngeutse Donfouet, T. Dimo et al. 2009. "Hypoglycemic and hypolipidemic effects of Ivingia gabonensis (Irvingiaceae) in diabetic rats," Pharmacologyonline, vol. 2, pp.957–962,
104. Ndifossap I. G. M., Frigerio F., Casimir M. et al. 2010, "Sclerocaryabirrea (Anacardiaceae) stem-bark extract corrects glycaemia in

diabetic rats and acts on β-cells by enhancing glucose stimulated insulin secretion," Journal of Endocrinology, vol. 205,no. 1, pp. 79–86,.
105. Patel D. K., Kumar R., Laloo D., &, Hemalatha. S. (2012), 'Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity' Asian Pacific Journal of Tropical Biomedicine, vol-2(5) pp. 411–420.
106. Gupta P., De A. Diabetes mellitus and its herbal treatment. Int. J. Res. Pharm. Biomed. Sci. 2012;3: 706–721.
107. Gupta R, Gupta GD. A review on plant Cordia obliqua Willd. (Clammy Cherry). Pharmacognosy Rev. 2015;9(18):127-31.
108. Gupta R, Gupta GD. Pharmacognostic, phytochemical and antioxidant studies on Cordia obliqua Willd. leaf. Inter J Pharm Sci Res. 2016;7(11):4669-76.
109. Gupta R, Gupta GD. Formulation Development and Evaluation of Anti-inflammatory Potential of Cordia obliqua Topical Gel on Animal Model. Pharmacog J. 2017;9(6)Suppl:s93-s8.
110. Dixit G, Misal G, Gulkari V and Upadhye K: Formulation and Evaluation of Polyherbal Gel for Anti - inflammatory Activity. Int J Pharm Sci Res. 2013; 4(2); 1186-1191
111. Fanun M. Formulation and characterization of microemulsions based on mixed nonionic surfactants and peppermint oil. Journal of Colloid and Interface Science 2010; 343(2): 496-503.
112. Goswami P, Choudhury A, Dey BK. Microemulsion – A Potential Carrier for Improved Bioavailability. International Journal of Pharmaceutical & Biological Archives 2019; 10(2): 69-77.
113. Changmai, A., Adhikari, A., Dey, K.B., (2019) Preparation and evaluation of Microemulsion containing clove oil and peppermint oil as active compound, Journal of Pharmaceutical and Scientific Innovation 8 (4), 155-158.
114. Sumalatha K et al. Design and In-vitro evaluation of nanogel containing mentha piperita American Journal of Biological and Pharmaceutical Research. 2014;1(3):136-13
115. Panner Selvam R, Kulkarni P. K, Naga Sravan Kumar Varma V. "Porous polystyrene spheres loaded self nano-emulsifying systems of rosuvastatin calcium", RSC Advances, 2015
116. Rahul S. Kalhapure, Krishnacharya G. Akamanchi. "Oleic acid based heterolipid synthesis, characterization and application in self-microemulsifying drug delivery system",International Journal of Pharmaceutics, 2012
117. S.N. Deepak., B. N. Vedha Hari (2013) Optimization, Development and evaluation of Microemulsion for the release of combination of Guaifenesin and Phenylephrine, Journal of Applied Pharmaceutical Science 3 (09), 048-056.
118. Gibaud S, Attivi D. Microemulsions for oral administration and their therapeutic applications. Expert Opin Drug Deliv 2012;9:937-51.
119. Bagley DM, Gardner JR, Holland G, Lewis RW, Regnier JF, Stringer DA, et al. Skin irritation: Reference chemicals data bank. Toxicol In Vitro 1996;10:1-6.
120. Swetha CH, Velmurugun S, Raju PN, Reddy GN. Formulation and evaluation of Clarithromycin topical gel. Inter J Drug Dev and Res. 2013;5(4):194-202
121. Barakoti H, Choudhury A, Dey BK. An Outlook for a Novel Approach: Self-Micro Emulsifying Drug Delivery System (SMEDDS). Research Journal of Pharmacy and Technology 2019; 12(4): 2055-64.
122. Ghosh PK, Murthy RSR. Microemulsion potential drug delivery system. Current Drug Delivery 2006; 3(2):167-80.
123. Lv X, Liu T, Ma H, Tian Y, Li L, Li Z, Tang Z. Preparation of essential oil-based microemulsions for improving the solubility, pH stability, photostability
124. Sudipta D, Haldar PK, Pramanik G. Formulation and evaluation of herbal gel containing Clerodendrum infortunatum leaves extract. Int J Pharmtech Res 2011;3:140-3.
125. Wood JH, Catacalos G, Liberman SV. Adaptation of commercial viscometers for special applications in pharmaceutical rheology – Severs extrusion rheometer. J Pharm Sci 1963;52:375-8.
126. Goyal S, Sharma P, Ramchandani V, Shrivastava SK, Dubey PK. Novel anti-inflammatory topical herbal gels containing Withania somnifera and Boswellia serrata. Int J Pharm Biol Sci Arch 2011;2:1087-94.
127. Mishra US, Murthey PN, Mishra D, Sahu K. Formulation and standerdisation of herbal gel containing methanolic extract of Calophyllum inophyllum. Am J Pharmtech Res 2011;1:276-89.
128. Jyothi D, Koland M, Priya S. Investigation of anti-inflammatory activity of ointments containing fenugreek extract. Asian J Pharm Clin Res 2014;7:66-9.
129. Dixit G, Misal G, Gulkari V, Upadhye K. Formulation and evaluation of polyherbal gel for anti-inflammatory activity. Int J Pharm Sci Res 2013;4:1186-91.
130. ICH Guidelines. Stability Testing of New Drug Substances and Products; 27 October, 1993.
131. Singh M, Mittal V. Formulation and evaluation of herbal gel containing ethanolic extract of ipomoea fistulosa. Int J Sci Res 2014;3:25-9
132. Nandgude, T.; Thube, R.; Jaiswal, N.; Deshmukh, P.; Chatap, V.; Hire, N. Formulation and evaluation of pH induced in situ nasal gel of salbutamol sulphate. Int. J. Pharm. Sci. Nanotechnol., v.1, n.2, p.177-83, 2008.
133. Hamzah M. Maswadeh, Sereen H Mohmmad, Abdulhalim A. Abdulatif. Invitro dissolution kinetic study of theophylline from hydrophilic and hydrophobic matrices. Acta Poloniae Pharmaceutica- Drug research 63(1) (2006) 63-67.
134. Kadimi US, Balasubramanian DR, Ganni UR, et al. (2007). In vitro studies on liposomal amphotericin
135. Murphy, C.T.; Mccarroll S.A.; Bargmann, C.I.; Fraser, A.; Kamath, R.S.; Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature, v.424, p.277-283, 2003.
136. Shah P, Swarnkar D. Development and characterization of microemulsion containing antihypertensive agent using factorial design. J Pharm Bioallied Sci 2012;4:69-70.
137. Dreher F, Walde P, Luisi PL, Elsner P. Human skin irritation studies of a lecithin microemulsion gel and of lecithin liposomes. Skin

Pharmacol 1996;9:124-9.
138. Zhua W, Guo C. Microemulsion-based hydrogel formulation of pencyclovir for topical delivery. Int J Pharm 2009;378:152-8.
139. Peeyush kumar, P.W. Ramteke, Avinash C. Pandey, Himanshu Pandey. "Evaluation of antifungal activity of blended cinnamon oil and usnic acid nanoemulsion using candidiasis and dermatophytosis models", Biocatalysis and Agricultural Biotechnology, 2019
140. Odds FC, Webster CE, Abbott AB. Antifungal relative inhibition factors: BAY 1-9139, bifonazole, butoconazole, isoconazole, itraconazole (R 51211), oxiconazole, sulconazole, terconazole and vibunazole compared in vitro with nine established antifungal agents. J Antimicrob Chemother 1984;14:105-14.
141. Chauhan, A., Sharma, P. K., Srivastava, P., Kumar, N. & Dudhe, R. "Plants Having 339 Potential Antidiabetic Activity", A Review. Der Pharma Lett. (2010): 340.
142. N. Stamp, "Out of the quagmire of plant defense hypotheses", Q Rev Biol. (2003), 78(1) :23-55.
143. Babu, V., Gangadevi, T., Subranabuab, A., "Antihyperglycemic effect of Cassia keinii leaf extract in glucose feed normal rats and alloxan-induced diabetic rats", Indian J. Pharmacol. (2002), 34: 409–415 495.
144. Patel SS, Shah RS, Goyal RK. "Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats", Indian J Exp Biol. (2009), 47(7):564–70.
145. Stavric, B. "Role of chemopreventers in human diet. Clin. Biochem", (1994) 27, 319– 322. DOI: 60510.1016/0009-9120(94)00039-5. 606
146. Chauhan, A., Sharma, P. K., Srivastava, P., Kumar, N. & Dudhe, R. "Plants Having 339 Potential Antidiabetic Activity", A Review. Der Pharma Lett. (2010): 340.
147. N. Stamp, "Out of the quagmire of plant defense hypotheses", Q Rev Biol. (2003), 78(1) :23-55.
148. Babu, V., Gangadevi, T., Subranabuab, A., "Antihyperglycemic effect of Cassia keinii leaf 493extract in glucose feed normal rats and alloxan-induced diabetic rats", Indian J. 494Pharmacol. (2002), 34: 409–415 495.
149. Stavric B., (1994) 'Role of chemopreventers in human diet'.Clin. Biochem, vol- 27, pp 319–322.
150. Chauhan,A.,Sharma,P.K.,Srivastava,P.,Kumar,N.&Dudhe,R.(2010),'PlantsHaving Potential Antidiabetic Activity A Review'. Der Pharma Lett.: pp-339-340
151. Yang, J. X., H. Wu., S. Ju., B. Zhu., D. Yan., Y. Wang., M. Hu. J., (2017) Preparation and evaluation of microemulsion-based transdermal delivery of Cistanche tubulosa phenylethanoid glycosides, Molecular Medicine Reports 15, 1109-1116.
152. Bowe P Whitney, Logan C Alan. Clinical implication of lipid peroxidation in acne vulgaris: old wine in new bottles. Lipids in Health and Disease 9(2010)140
153. Krautheim Andrea, Gollnick M P Harald. Acne: Topicaltreatment.ClinicsinDermatol.22(2004)398-407.
154. Das Kuntal, Dang Raman, Machale U Manjunath.Formulation and evaluation of a novel herbal gel of Stevia extract. Iranian J Dermatol.12 (2009)117-122
155. Chithra V., Leelamma S. Coriandrum sativum changes the levels of lipid peroxides and activity of antioxidant enzymes in experimental animals. Indian J Biochemistry and Biophysics36(1999) 59-61
156. Matasyoh J.C.,Maiyo Z.C.Chemical composition and antimicrobial activity of the essential oil of coriandrum sativum. Food Chemistry113(1999)526-529.
157. Deepti Kaushalkumar Jani, Sunita Goswami. "Antidiabetic activity of Cassia angustifolia Vahl. and Raphanus sativus Linn. leaf extracts", Journal of Traditional and Complementary Medicine, 2020
158. Changez M, Varshney M. Aerosol-OT nanoemulsions as transdermal carriers of tetracaine hydrochloride. Drug Dev. Ind. Pharm. 2000; 26: 507–512.
159. Sharififar, S.; Dehghn-nudeh, G.; Mirtajaldini, M. Major flavonoids with antioxidant activity from Teucrium polium L. Food Chem., v.112, n.4, p.885-888, 2009
160. Hongliang Zhang, Qiuyan Huang, Zhenguang Huang, Taotao Liu, Yuanhong Li. "Preparation and physicochemical properties of chitosan broadleaf holly leaf nanoparticles", International Journal of Pharmaceutics, 2015.
161. Mncwangi, Nontobeko, Weiyang Chen, Ilze Vermaak, Alvaro M. Viljoen, and Nigel Gericke. "Devil's Claw—A review of the ethnobotany, phytochemistry and biological activity of Harpagophytum procumbens", Journal of Ethnopharmacology, 2012.