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Design, Development and Evaluation of Herbal Transdermal Patches for Anti-Inflammatory Activity

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ABSTRACT

In this study, various transdermal matrix patches containing Commiphora mukul of variable combination of ethyl cellulose/polyethylene glycol with enhancer (menthol: limonene) were prepared. The prepared patches were studied with respect to physicochemical characters, drug-excipient interaction, dissolution, skin permeation, stability and *in vivo* anti-inflammatory studies. The combination of ethyl cellulose and polyethylene glycol produces smooth flexible films. Dissolution and *in vitro* skin permeation studies revealed that the cumulative amount of drug permeated was decreased as the polyethylene content of the film increased. The film containing enhancer shows greater release as compared to film containing no enhancer. Based on *in vitro* skin permeation studies, CM4 [PEG/EC, 1:5, menthol (*36 ug*): limonene (*36 ul*)], was found to be better formulation as it released a maximum amount of drug. In stability studies, all the formulations were stable with respect to physical properties up to 45 days.

Keywords: Transdermal patches, Commiphora mukul, Ethyl cellulose/polyethylene glycol, Enhancer.

INTRODUTION

Discovering a new medicine is a very expensive and time-consuming undertaking. However, re-designing the modules and means to transport medicine into the body is a less demanding and more lucrative task. The design of dosage form, whether a tablet, an injection or a patch; to deliver the right amount of medicine at the right time to the right target site becomes complicated if each medication were to be delivered in an optimal and preferred manner to the individual patient. The medication may not be absorbed if it is released too slowly. If it is delivered too rapidly, the patient may suffer untoward effects and its desired effects may not last if needed. If patient is expected to take the medicine more than two times a day, compliance will be adversely affected. One of the solutions developed is transdermal drug delivery system which can deliver medicines via the skin portal to systemic circulation at a predetermined rate and maintain clinically effective concentration over a prolonged period. This route of drug administration avoids the hazards and discomfort associated with parental therapy and improves patient compliance, as it is easy to apply a patch [1].

Despite the small number of drugs currently delivered via this route, it is estimated that worldwide market revenues for transdermal products are US $ 3B, shared between the USA at 56%, Europe 32% and Japan at 7%. In a recent market report, it was suggested that the growth rate for transdermal delivery system will increase 12% annually (Benson AE, 2005) [2]. Transdermal products for cardiovascular disease, Parkinson's

disease, Alzheimer's disease, inflammation, pain, arthritis, depression, anxiety, attention deficit hyperactivity disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal bone loss, weight loss etc [2].

Inflammation (Latin, *inflammatio*, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally tightly regulated by the body.

A good number of drugs are commonly used in the Indian system of medicine for the treatment of inflammation. Out of them, *Commiphora mukul* (Guggul gum or Guggul) has been in use for long time arthritis. It has been associated with gastric upset as a major adverse effect and has to be administered 2-3 times a day [3]. The transdermal patch delivery system may be an attractive choice of an alternative route of administration of this drug. It can be a good candidate for transdermal drug delivery system because it possesses characteristic properties required for TDDS such as sparingly soluble in water, smaller dose, lower melting point and molecular weight less than 700 Dalton. Moreover, the logarithmic value of the partition coefficient of the drug on octanol-water system (3.464), which is nearer to 3, is required for the transdermal patch delivery system [4,5]. Presently no scientific reports are available on the formulation of transdermal drug delivery system of *Commiphora mukul* for the treatment of inflammation. Hence in this study an attempt has been made to prepare and evaluate the transdermal drug delivery system of *Commiphora mukul.*

MATERIALS AND METHODS

Standard guggulsterone Z was procured from SPIC Pharmaceutical, Maraimalal Nagar, Tamil Nadu. *Commiphora mukul* dry extract was supplied by Nicholas Piramal as gift sample. Menthol (Loba Chemie Pvt. Ltd.), Ethyl cellulose and Dibutyl phthalate.Mumbai), Poly isobutylene and Limonene (National chemicals, Vadodara), Poly vinyl alcohol, M. W. 1, 2500 (Qualigens, Mumbai), Polyetylene glycol 4000 (Suvidhinath Laboratories, Baroda), 1-Butanol (Sisco Research laboratories, Mumbai), Chloroform, Methanol (HPLC Garde), 2-Propanol (HPLC grade), Acetonitrile (HPLC Grade) of (Merck, Mumbai) was used. All other reagents and solvents used were of analytical grade.

The following instruments and equipment were used for the study. HPLC (SHIMADZU SCL-10A VP), UV-VIS Spectrophotometer (SHIMADZU UV-1650 PC), Weighing balance (Adventure, OHAUS Corp, NJ, USA) Cyclomixer (CM 101, Remi equipment, Mumbai), Hot air oven (Osworld, Mumbai), Ring water bath (Growell Instruments, Bangalore), Fast clean ultrasonic cleaner (Enertech Electronics Pvt. Ltd.), Orbital shaking incubator (CIS-24, Remi equipments, Mumbai), Membrane filter equipment (Millipore, Bangalore), Membrane filter (ALL Pharmalab filtration pvt.ltd, Mumbai), Adhesive tape USP (Leukoplast, Goa), Millipore filter (HVLP01300, Millipore, Bangalore), Magnetic stirrer (2 -MLH, Remi Instruments, Mumbai), Paleothermometer (UGO Basile 7140).

Analytical method for determination of guggulsterone Z

Analytical (HPLC) method

HPLC system: SHIMADZU; SCL-10A *VP*

Pumps: LC-10AT *VP*

Column: Octadecyl column; 250 mm × 4.6 mm, 5 µm; Phenomenex, Cheshire, UK)

Flow rate: 1.0 ml

Mobile phase: Acetonitrile: Water (75:25)

Detecting wavelength: 242 nm

Chromatographic data was acquired using Class VP software

Calibration curve of guggulsterone Z

Preparation of stock and sample solutions

25 mg of guggulsterone Z was dissolved in 10 ml of methanol, serving as stock solution (250 µg/ml). Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.0 of stock solution were pipetted into 10.0 ml volumetric flask and the volume was made up to 10 ml with mobile phase [Acetonitrile: Water (75:25)] to get the concentration of 2.5, 5.0, 10.0, 20.0 and 25 µg/ml respectively.

Preparation of phosphate buffer saline (pH 7.4) I. P. 1996

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. The pH was adjusted to 7.4

using *O*-phosphoric acid 0.01M solution.

Pre-formulation studies

Solubility study

The solubility studies were performed in phosphate buffer solution, 7.4 and 10% methanolic phosphate buffer saline by adding excess amounts of drug in each case and keeping the excess drug containing phosphate buffer flasks on a water bath shaker for 24 h at 32°C. After that solutions were analyzed by HPLC [6].

Partition coefficient

The octanol/water partition coefficient (Kow) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system.

Estimation of guugulsterone Z in extract by HPTLC

Kow = Concentration in octanol phase / Concentration in aqueous phase

Sample preparation

10 mg of *Commiphra mukul* extract was dissolved in methanol and transferred to a 10 ml volumetric flask (1 mg/ml). The volume was made up to the mark with methanol. This solution was further used for HPTLC estimation.

Standard preparation

250 µg/ml solution of guggulsterone Z reference standard was prepared in methanol.

HPTLC estimation

The test sample was applied in volumes of 4, 6, 8, 10, 14 and 16 µl on TLC aluminum plates pre coated with silica gel. The standard solution was applied in volumes of 4, 6 and 8 µl. The chromatogram was developed in toluene-acetone (9:1) and scanned at 245 nm. The amount of guggulosterone Z was calculated by following formula.

$$\textbf{\% purity} = \frac{\text{Conc. of standard} \times \text{area of test} \times \text{volume of test sample}}{\text{Conc. of test} \times \text{area of standard} \times \text{volume of standard}}$$

Estimation of guggulsterone Z by HPLC

Calibration curve of guggulsterone Z (new column)

Preparation of stock and standard solutions

25 mg of guggulsterone Z was dissolved in 10 ml of methanol, serving as stock solution (250 µg/ml). Aliquots of 0.1, 0.2, 0.4, 0.5,0.8 and 1.0 of stock solution were pipetted into 10.0 ml volumetric flask and the volume was made up to 10 ml with mobile phase [Acetonitrile: Water (75:25)] to get the concentration of 2.5, 5.0, 10.0, 12.5, 20.0 and 25 µg/ml respectively.

Preparation of sample

10 mg and 5 mg of *Commiphra mukul* extract was dissolved in methanol and transferred to a 10 ml volumetric flask. The volume was made up to the mark with methanol. From the above solution 1 ml was pipetted into 10 ml volumetric flask and the volume was made up to 10 ml with mobile phase [Acetonitrile: Water (75:25)] to get the concentration of 100 and 50 µg/ml respectively. Samples were estimated by HPLC

Formulation development

Development of patch

Matrix type transdermal patches containing *Commiphora mukul extract* were prepared using the different ratios of polyethylene glycol and ethyl cellulose by solvent evaporation technique in flat bottom petridish. On the flat bottom of petridish backing membrane was casted by pouring 3% w/v polyvinyl alcohol solution followed by drying at 60°C for 6 h. The polymers weighed in requisite ratio and were dissolved in chloroform. Di-n-Butyl phthalate (20% w/w of polymer composition) was added to the solution. The drug equivalent to 50% w/w of the total weight of polymers was added, in the homogenous dispersion, by slow stirring with mechanical stirrer. The uniform dispersion was casted on the PVA backing membrane casted earlier and dried at 40°C for 16 h. The backing membrane was then glued to gummy tape keeping matrix type upward. This was the final shape of the formulation (Tables 1 and 2).

Table 1: Composition of various film formulations.

Formulation	Ratio of PEG /EC	Total weight of PEG /EC (mg)	Chloroform (ml)	DBT (with respect to polymer weight)	Menthol: Limonene (µl:mg)	Drug (with respect to polymer weight)
CM1	1:5	480	20	20%	-	50%
CM2	1:4	480	20	20%	-	50%
CM3	1:3	480	20	20%	-	50%
CM4	1:5	480	20	20%	36:36	50%
CM5	1:4	480	20	20%	36:36	50%
CM6	1:3	480	20	20%	36:36	50%

PEG: Polyethylene glycol 4000, EC: Ethyl cellulose, DBT: Dibutylpthalate.

Table 2: Patch parameters.

Total Internal Diameter	70 mm
Patch diameter	28 mm
Total area	3846.5 mm^2
Patch area	615.44 mm^2

Evaluation of formulation

The films were evaluated for the following parameters

Drug content by HPLC

Patches (2.8 cm^2) were cut and added to 10 ml volumetric flask and volume was made to 10 ml with methanol and kept for sonication for half an hour. Then, the subsequent dilution was made by mobile phase. Samples were estimated by HPLC by using standard calibration curve. The procedure was repeated in triplicate for each formulation [7].

Weight variation studies

Six films from each batch were weighed individually and the average weight was calculated.

Thickness

The thickness of film was measured by using screw gauge, with a least count of 0.01 mm. Thickness was measured at five different points on the film and average of five readings was taken [8].

Folding endurance

Folding endurance was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value [9].

Percentage of moisture content

The films were weighed individually and kept in a desiccators containing activated silica at room temperature for 24 hours. Individual films were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight [10].

Percentage of moisture uptake

A weighed film kept in desiccators at room temperature for 24 hours was taken out and exposed to 84% relative humidity (a saturated solution of sodium chloride) in desiccators until a constant weight for the film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight [9].

Drug-excipient interaction studies

HPLC studies

Drug- excipient interaction study was carried out by HPLC. Drug was mixed with each excipient in equal proportion. If there is no change in both retention time and area then there is no interaction [6].

HPTLC studies

High performance thin layer chromatography (HPTLC) data's were taken on a Camag instrument to find out the drug-excipient interaction used in the formulation. HPTLC chromatogram of the drug, excipients and composition of final formulation were obtained by using toluene: acetone (9:1) as mobile phase on precoated silica aluminum plates. The plate was scanned densitometrically at 254 nm by comparing the integrated *Rf* with those of standard.

***In vitro* release**

Dissolution studies

Herbal transdermal film measuring 2.8 cm^2 were subjected to *in vitro* diffusion testing using Keshary-Chien diffusion cell. Mesh (60) was clamped between the donor and receptor compartments and the film was placed over the mesh. The receptor compartment contained Phosphate buffer /methanol (9:1). The amount of drugs diffusing into the receptor compartment across the mesh was determined by withdrawing 0.5 ml samples over the duration of experiment and an equivalent amount of diffusion medium was added to the receptor compartment to maintain a constant volume. The samples were filtered through injection millipore filter of 0.45 µ. These samples were analyzed by HPLC. The cumulative amount of drug permeating through the mesh was then calculated [10,11].

***In vitro* skin permeation study**

In vitro permeation studies were carried out by using diffusion cell. Full thickness abdominal skin of male wistar rats weighing 200 to 250 g was used. Hair from abdominal region was carefully removed by sharp edge blade, 12 h before starting the experiment. The dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissue or blood vessels and equilibrated for an hour in buffer pH 7.4 before starting the experiment and treated skin was used for the experiment.

Herbal transdermal films measuring 2.8 cm^2 were subjected to *in vitro* diffusion testing using Keshary-Chien diffusion cell. Suitably prepared hairless rat skin was clamped between the donor and receptor compartments and the film was placed over the skin. The receptor compartment contained Phosphate buffer /methanol (9:1). All studies were performed at 37°C ± 1°C, at 50 rpm, containing 100 ml of phosphate buffer [6].

The rest of the procedure was same as that of dissolution studies. Animals used in this study were approved by Institutional Animal Ethics Committee, letter no. IAEC/KMC/07/2007-2008 dated 22 Nov 2007.

Stability studies

The prepared films were wrapped in aluminum foil. The aluminum foils were placed in a stability chamber whose temperature was maintained at 40°C ± 2°C at 75% RH for 45 days. Then, films were withdrawn and evaluated for physical parameters like color, thickness and weight of the films. All storage conditions were maintained as per ICH guidelines.

Primary skin irritation study

Three wistar albino rats of either sex weighing 200-250 g were used for the test. The intact skin was used. The skin from the back of each rat was depilated 24 hours prior to application of the patch. Two areas of the back of each rat, approximately 10 cm apart were designated for the position of the patches. One area was used for application of plain polymeric patch and the other was used for drug patch. The animals were immobilized using rabbit holder during 24 hours exposure. Upon removal of the patches, the resulting reaction was evaluated using weighed scores. Reading was also made after 72 hours and the final scores represent an average of the 24 and 72-hour reading **(**Table 3) [12].

Table 3: Evaluation of skin reactions.

Skin reaction	Score
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema	4
Total possible erythema score	4

***In vivo* study**

The anti-inflammatory activity and sustaining action of the drug loaded matrix patches were evaluated using the 'carrageenan' induced hind paw edema' method developed by Winter. Young male rats, weighing approximately 175 g, were taken for the experiment, divided into four groups, each containing four rats. The rats were kept on fasting overnight. The back sides of rats were shaved 12 h before starting the experiment. Patches were applied on the backs of all the animals (except control group) half an hour before sub plantar injection of carrageenan in the right paw. Paw edema was induced by injecting 0.1 ml of a 2% w/v homogenous suspension of carrageenan in double distilled water. The volume of injected paws measured immediately (0 h) and 1, 2, 3, 4 and 5 h after injection using plethysometer. The amount of paw swelling was determined time to time and expressed as percent edema relative to initial (0 min) hind paw volume. Percentage inhibition of edema produced by each patch treated group was calculated against the respective control group using the following formula [6].

$$\% \text{ inhibition} = \frac{\text{Edema (control) - Edema (drug)}}{\text{Edema (control)}} \times 100$$

Statistical analysis

The data was analyzed using One way Anova followed by Post Hoc Scheffe's Test using SPSS computer software version 7.5. Level of significance was fixed at 0.05.

RESULTS AND DISCUSSION

Pre-formulation studies

Solubility studies

An attempt has been made to find out which media was able to maintain sink conditions in dissolution as well as in permeation studies. Results indicate that the drug was more soluble in 10 % methanolic phosphate buffer saline than plane phosphate buffer saline. The 10 % methanolic phosphate buffer was chosen as the dissolution and permeation media because sufficient amount of drug dissolved in it. (3 times the drug incorporated in patch), which is necessary to maintain sink condition (Table 4).

Table 4: Solubility studies.

Sr. No.	Medium	Concentration of drug µg/ml	Concentration of drug µg / 100 ml
1	Phosphate buffer saline pH 7.4	0.9126	912.6053
2	10% methanolic phosphate buffer saline pH 7.4	3.4422	3442.2086

Partition coefficient determination

Octanol and water are considered to be the standard system to determine the drug partition coefficient. The partitioned coefficient was determined using the formula shown in experimental section. The value of partitioned coefficient was experimentally found to be 2.244. The results indicates the lipophilic nature of drug, which fulfills the formulating it into a transdermal patch. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

Estimation of guugulsterone Z in extract by HPTLC

Result: The % content of guggulsterone Z in the extract was found to be approximately 3.4 % w/w (Figures 1-3).

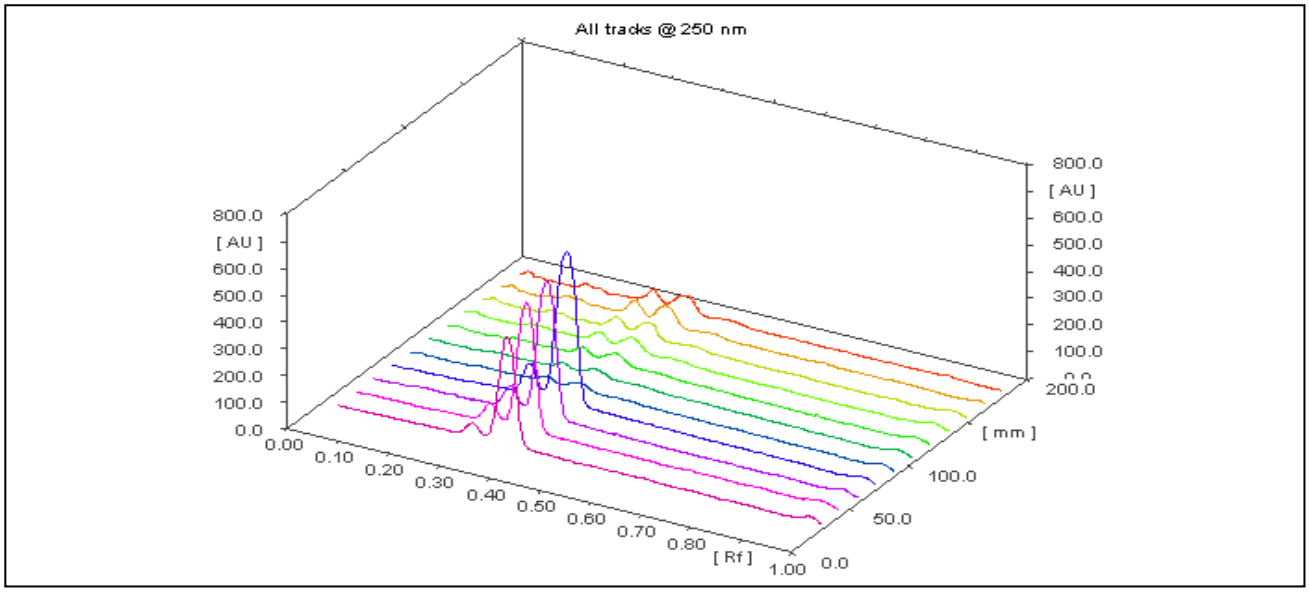


Figure 1: 3D Chromatogram image at 250 nm.

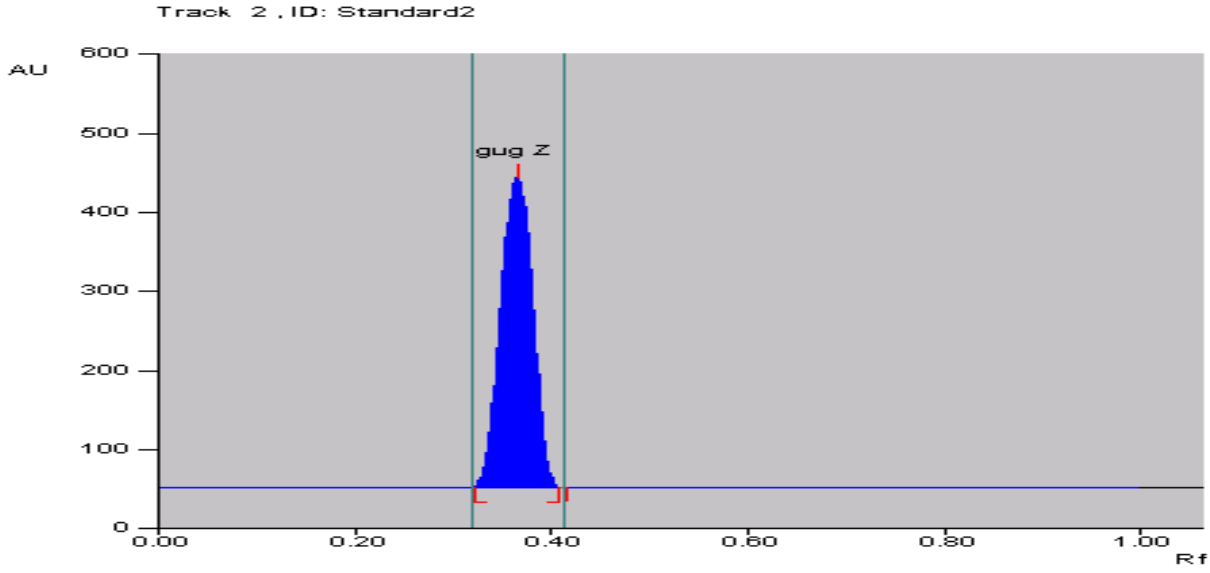


Figure 2: HPTLC chromatogram of standard guggulsterone Z *Rf* = 0.36.

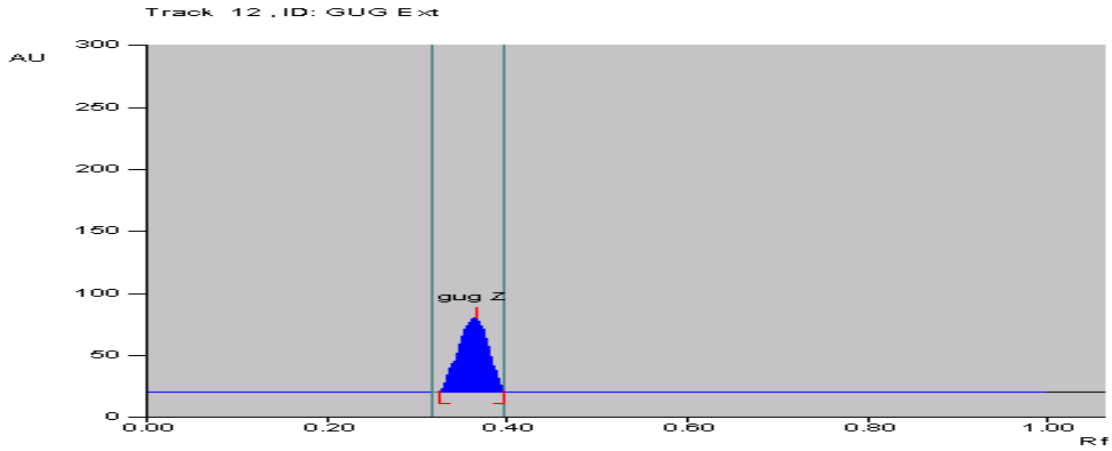


Figure 3: HPTLC chromatogram of extract Rf = 0.37.

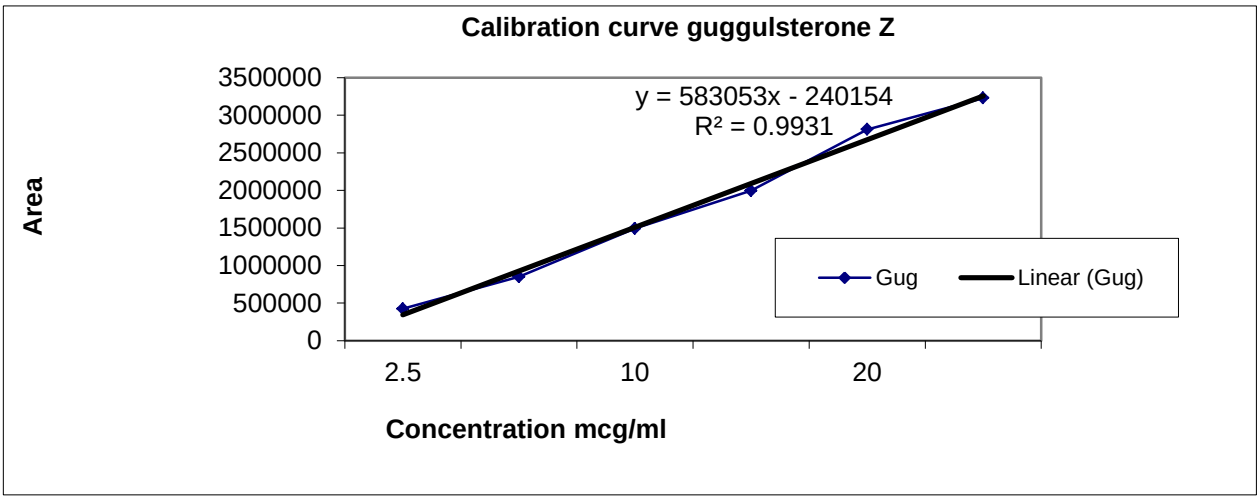


Figure 4 : Calibration cruve of guggulsterone Z (new column).

Estimation of guugulsterone Z in extract by HPLC

Calibration curve was plotted between the concentration and area (Figure 4).

Result: The % content of guggulsterone Z in the extract was found to be approximately 3.49% w/w (Table 5).

Table 5 : Result of guggulsterone present in extract.

Sr. No.	Extract conc. (µg/ml)	Area	Guggulsterone Z content (µg/ml)	Amount of gug. Z in extract
1	100	179496	3.490	3.490%
2	50	78066	1.750	3.5%

Formulation development

Ethyl cellulose and polyethylene glycol 4000 were used for the preparation of polymer. The films obtained were smooth and flexible.

Evaluation of formulation

The formulated films were characterized for various parameters such as weight variation, thickness and drug content. These are essential parameters for the evaluation of the dosage form in order to achieve a formulation with uniformity and consistency within a batch. The results of weight variation, thickness and drug content as shown in Table 6.

Table 6: Results of weight variation, thickness and drug content.

Formulation	Weight of the patch / 2.8 cm^2	Thickness	Drug content (%)
CM1	119.33 ± 1.53	0.132 ± 0.001	96.94 ± 0.53
CM2	121.5 ± 1.0	0.143 ± 0.008	86.75 ± 0.64
CM3	120.33 ± 1.53	0.138 ± 0.010	81.69 ± 3.09
CM4	120.0 ± 2.64	0.142 ± 0.007	98.29 ± 0.42
CM5	120.0 ± 2.0	0.145 ± 0.007	80.76 ± 0.65
CM6	120.66 ± 1.53	0.136 ± 0.012	81.77 ± 4.36

All values are presented as Mean ± S.D.; n=3

The films exhibited uniform weight and thickness among the various batches. The uniformity of weight and thickness indicates that the polymeric solution of the drug is well dispersed on flat surface. However, little variation in weight and thickness observed in different formulation may attribute to the variation in polymeric content. All formulation exhibited slight variation in drug content ranging from 80.86 % to 98.29%.

Percentage of moisture content and percentage of moisture uptake

The results of moisture content and moisture uptake are shown in Table 7 and Figure 5.

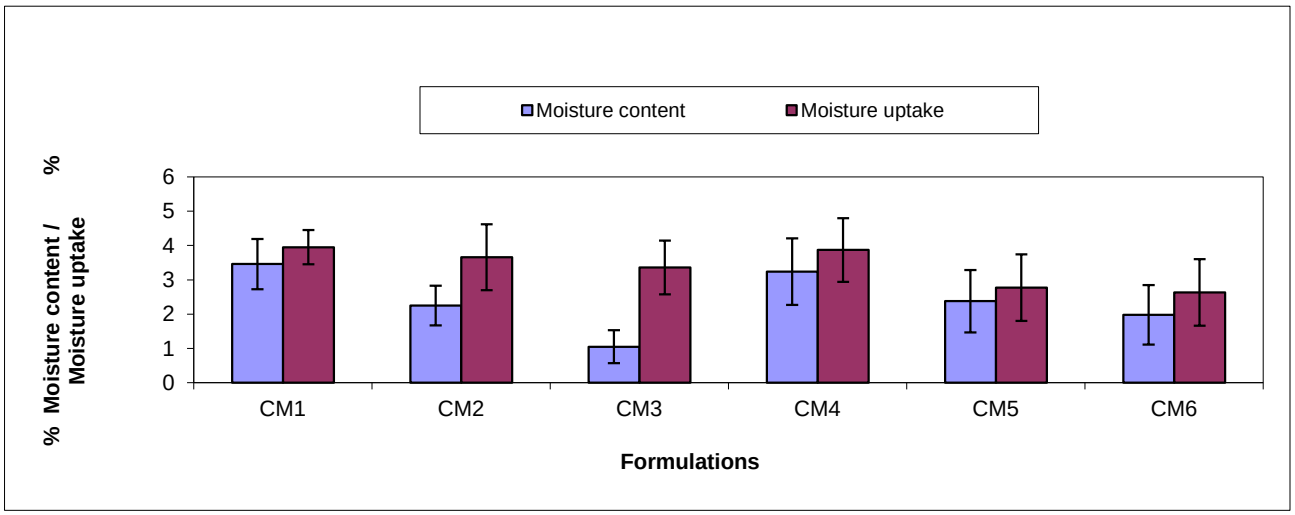


Figure 5: Percentage of moisture content and moisture uptake from different formulations.

Table 7: Results of moisture content and moisture uptake.

Formulation	Moisture content	Moisture uptake
CM1	3.46 ± 0.73	3.95 ± 0.50
CM2	2.25 ± 0.58	3.66 ± 0.96
CM3	1.05 ± 0.48	3.36 ± 0.78
CM4	3.24 ± 0.97	3.87 ± 0.93
CM5	2.38 ± 0.91	2.77 ± 0.97
CM6	1.98 ± 0.87	2.63 ± 0.97
All Values are expressed as Mean ± S.D. n=3		

The moisture content and moisture uptake was found to be decreasing with the increasing quantity of PEG, which can attribute non-hygroscopic nature of PEG, and it was also found that the moisture content was little higher in formulation containing the enhancer (menthol: limonene) compare to formulation containing no enhancer. The small moisture content in the formulation helps them to remain stable and prevent from being a completely dried and brittle. A low moisture uptake protects the material from microbial contamination and bulkiness of the patch [6].

Folding endurance

The result of folding endurance was shown in Table 8.

Table 8: Results of folding endurance.

Formulation	Folding endurance value
CM1	9.33 ± 1.52
CM2	14.33 ± 1
CM3	21 ± 2
CM4	47.66 ± 3.22
CM5	>50
CM6	>50

All values are expressed as Mean ± S.D. n=3

Table 9: Determination of drug-excipient interaction using HPTLC.

Sr. No.	Substance	*Rf*	Area
1	Reference standard	0.44	8284.7
2	Extract	0.44	6632.3
3	Formulation	0.42	6609.3
4	Blank patch	-	-

Finding shows that plasticity of formulation increases with the increasing quantity of PEG and decreasing quantity of ethyl cellulose. The formulation containing enhancer (CM4, CM5, and CM6) having more folding endurance value, hence it can be concluded that the menthol: limonene imparts extra plasticity to the formulation.

Drug-excipient interaction studies

HPLC studies

HPLC studies were performed to assess any interaction between the drug and excipients. The results are shown in Figures 6-9.

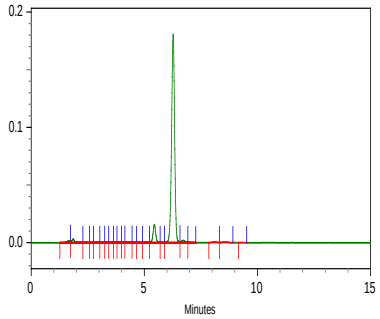


Figure 6: HPLC chromatogram of standard guggulsterone Z extract.

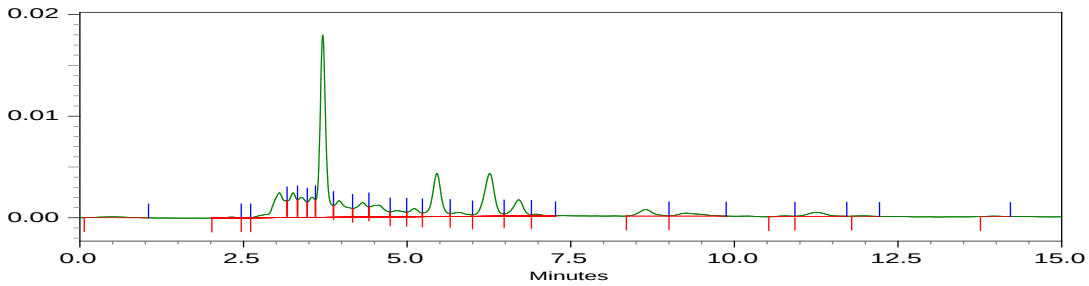


Figure 7: HPLC chromatogram of extract.

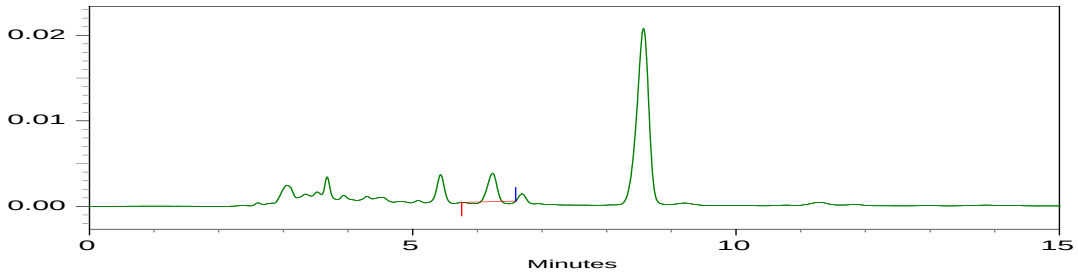


Figure 8: HPLC chromatogram of formulation.

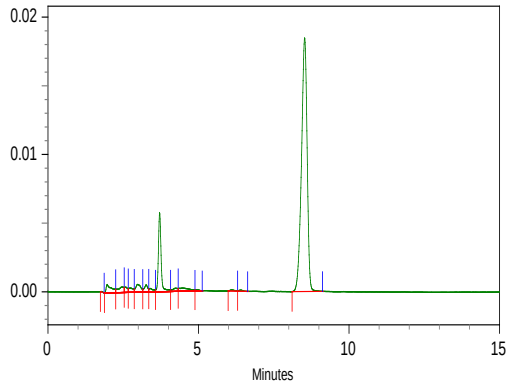


Figure 9: HPLC chromatogram of blank patch.

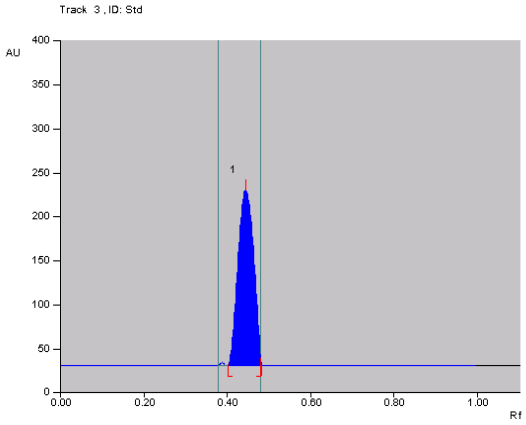


Figure 10: HPTLC chromatogram of standard guggulsterone Z.

The data obtained from above figures suggested that there was no interaction between the drug and excipient as the retention time of drug and area were similar for both formulations and standard. It was also noticed that there was no interference of excipient on the retention time of drug.

HPTLC studies

HPLC studies were performed to assess any interaction between the drug and excipients. The results are shown in Figures 10-14 and Table 9.

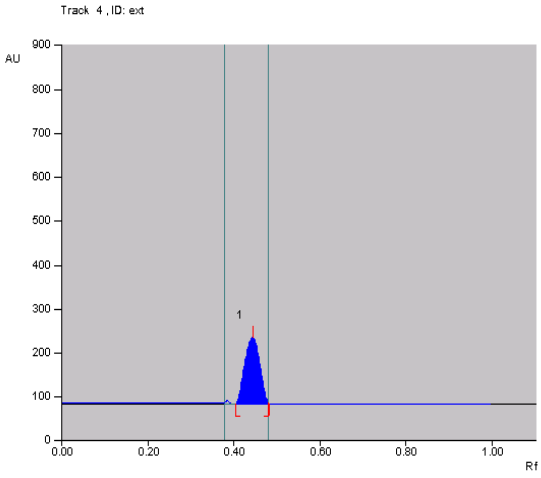


Figure 11: HPTLC chromatogram of extract.

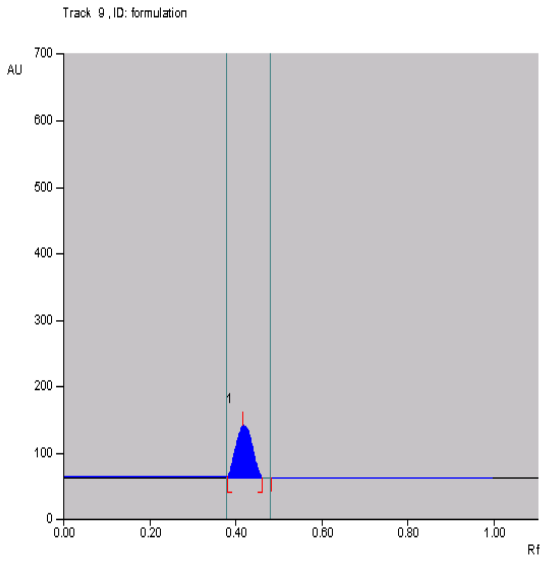


Figure 12: HPTLC chromatogram of formulation.

The data obtained suggested that there was no interaction between the drug and the excipients since the *Rf* values and area of the standard, extract and formulation was found to be almost similar.

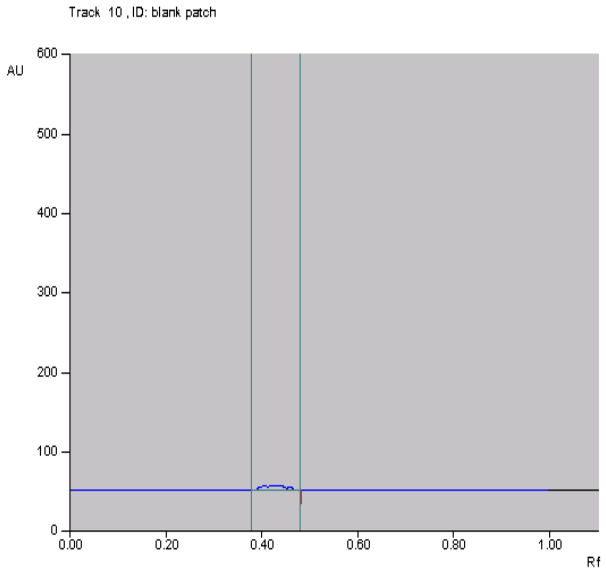
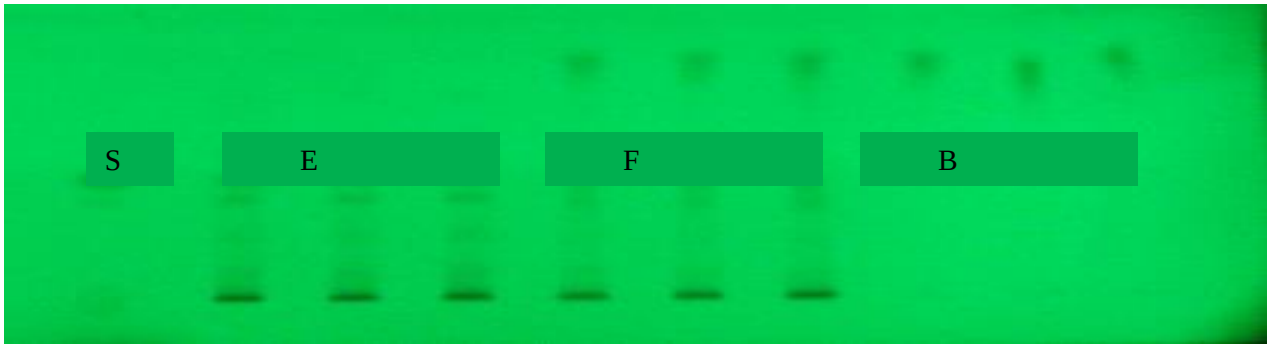


Figure 13: HPTLC chromatogram of blank patch.



S: standard, E: extract, F: formulation B: blank formulation

Figure 14: Photo documentation at 254 nm.

***In vitro* release**

In vitro dissolution studies

Dissolution studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. Release studies are required for predicting the reproducibility of rate and duration of drug release. The importance of polymer dissolution on drug release from matrices has been known for ensuring the sustained release performance [13]. Dissolution studies for different formulation were performed using Keshary-Chien diffusion cell using 10% methanolic phosphate buffer, pH 7.4, as dissolution medium at 32°C [6]. The results are shown in Table 10 and Figure 15.

Table 10: In *vitro* dissolution studies of *Commiphora mukul* extract.

Hour	Cumulative % of drug release					
	CM1	CM4	CM2	CM5	CM3	CM6
0	0	0	0	0	0	0
1	9.041	13.117	8.775	12.126	5.590	6.530
2	16.640	22.368	14.649	19.286	10.790	11.637
3	23.651	32.023	20.516	27.965	16.467	18.420
4	30.257	40.242	27.970	33.951	22.590	24.072
5	35.464	46.186	31.124	37.546	27.890	29.352
6	40.852	51.615	36.473	43.049	31.828	35.023
7	46.183	54.531	41.029	48.315	35.739	38.927
8	50.711	57.529	45.107	52.127	38.476	41.424
9	53.570	60.492	48.737	54.870	40.747	44.221
10	55.745	62.252	50.951	58.218	41.725	46.192
11	57.299	63.887	52.249	60.163	42.486	47.046
12	58.951	64.925	54.565	61.110	44.332	49.239

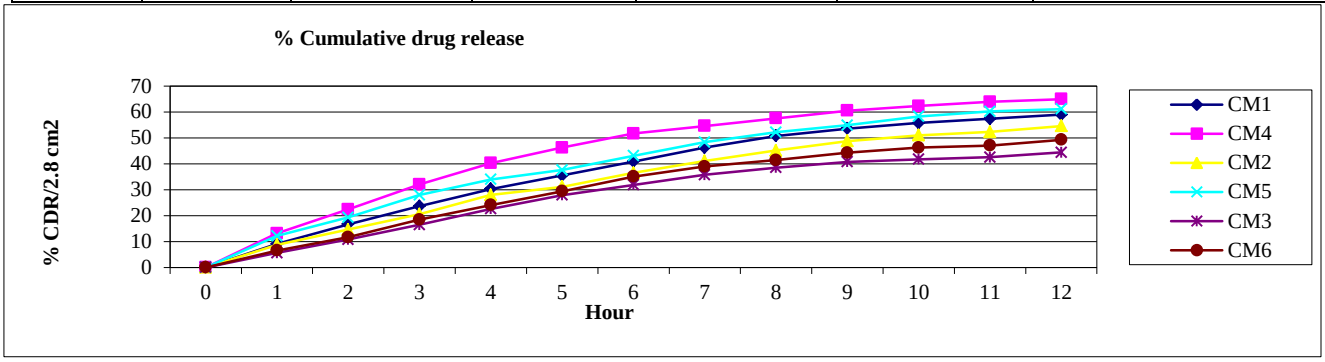


Figure 15: In *vitro* dissolution studies of *Commiphora mukul* extract.

Maximum percentage of drug release (64.935) was found for the formulation CM4 (EC/PEG, 1:5, Menthol: Limonene, 10% of total weight) and minimum percentage of drug release (44.332%) was observed for the formulation CM3 (EC/PEG, 1:3).

The cumulative percentage of drug dissolution from the various formulations was found to be.

CM1: 59.591

CM2: 54.565

CM3: 44.332

CM4: 64.925

CM5: 61.110

CM6: 49.239

The cumulative % of drug permeated from different formulations was increased in the following order.

CM4> CM5> CM1> CM2> CM6 C CM3

The drug release from all the films was rapid in the initial hours (up to 6 hours), which could be due to the presence of drug on the surface of films. Later, the drug was released slowly from the patches.

It was observed that as the concentration of hydrophilic polymer, PEG increases in the formulation, the rate of dissolution decreases subsequently. This may be due to the film forming property of PEG, which may form on the surface of film and delay the drug release. It has been also observed that the drug release was greater in formulations containing enhancer in comparison to formulations containing no enhancer as shown in Table 11.

Table 11: Result of enhancement ratio.

Formulation without enhancer		Formulation with enhancer		Enhancement ratio*
CM1	59.591	CM4	64.925	1.089
CM2	54.565	CM5	61.110	1.119
CM3	44.332	CM6	49.239	1.110

* Enhancement ratio is calculated by dividing the flux with enhancer with that of flux without enhancer (Benson, 2005).

Hence, it may conclude that the addition the enhancer (menthol: limonene) increases the drug release. Menthol and limonene increases the drug release by changing the physicochemical property of drug such as melting point [2] and there are much literature available which state that it act by enhancing the drug permeation by possible reversible disruption of the intercellular lipid domain and increased the diffusion coefficient of the drug in the skin.

***In vitro* skin permeation study**

Release of drug from transdermal patches is controlled by the chemical properties of drug and delivery form as well as the physiological and physicochemical properties of the biological membrane [14]. The *in vitro* permeation studies are predictive of *in vivo* performance of a drug [15]. Matrix or monolithic transdermal drug delivery devices are used when the rate of drug permeation through the stratum corneum is the rate-limiting step for the drug absorption. The implication of skin permeation of drug on release rate profiles of the experimental formulation should not be ignored because; the skin is known to have substantial role in variation of release kinetics [16].

Two formulations CM5, CM4 were selected for the *in vitro* skin permeation studies based on the result obtained *in vitro* dissolution studies. The results are shown in the Table 12 and Figure 16.

Table 12: In *vitro* skin permeation studies of *Commiphora mukul* extract.

Hour	Cumulative amount release (µg / 2.8 cm^2)	
	CM5	CM4
0	0	0
1	13.550	15.971
2	27.004	30.290
3	36.731	38.263
4	41.927	44.0395
5	44.690	49.210
6	49.813	56.396
7	55.086	59.194
8	58.427	62.427
10	63.160	70.572
12	66.601	73.626

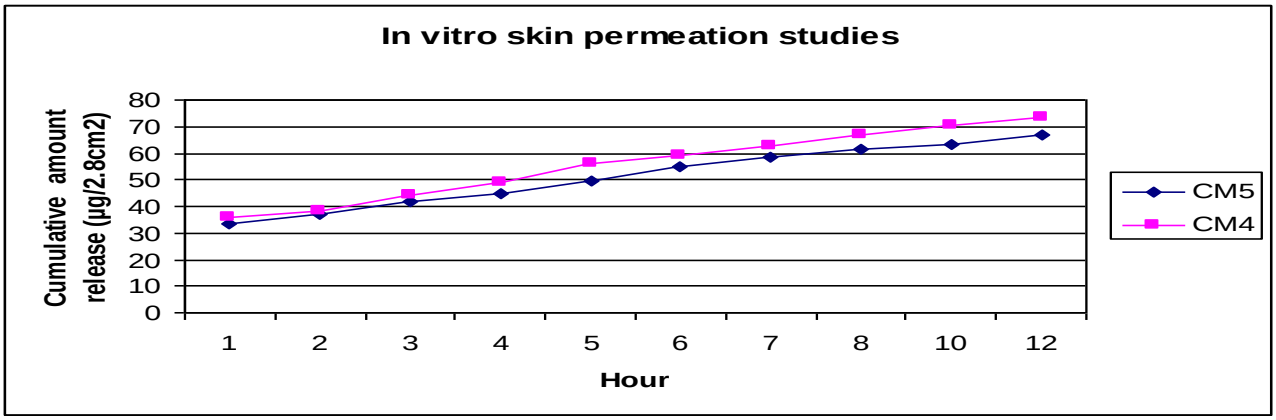


Figure 16: In *vitro* skin permeation studies of *Commiphora mukul* extract.

Table 12: In *vitro* skin permeation studies of Commiphora mukul extract.

Formulations	Physical characteristics (color, weight, thickness)			
	0 days	15 days	30 days	45 days
CM1	+++	+++	+++	+++
CM2	+++	+++	+++	+++
CM3	+++	+++	+++	+++
CM4	+++	+++	+++	+++
CM5	+++	+++	+++	+++
CM6	+++	+++	+++	+++

The above results indicate that the drug permeation was greater in formulation containing enhancer due to fact that the terpenes act by disrupting the lipid structure of the stratum corneum [2]. It was found that the drug permeation from rat skin was less as compared to drug release from *in vitro* dissolution studies.

Drug release kinetics

First order

It is observed that the plot does not shows linear regression as the R^2 was found in the range of 0.561- 0.748. It means that release pattern does not follow first order kinetics

Zero order Kinetics

It is observed that the plot shows linear regression as the R^2 was found in the range of 0.931 - 0.940. It indicates that drug release follows zero order kinetics.

Higuchi model

The drug release pattern does not show linear regression (and not following diffusion kinetics mechanism.

Above all finding shows that the drug releases from different formulations may follow zero order kinetics (Table 13).

Table 13: Drug release kinetics parameter of permeation studies through rat skin.

Formulation	First order plot		Higuchi plot		Zero order plot	
	R^2	K	R^2	K	R^2	K
CM5	0.748	0.622	0.748	0.622	0.931	6.093
CM4	0.561	0.121	0.561	0.121	0.940	6.701

Stability studies

The results of stability studies are shown in Table 14.

Table 14: Stability studies of *Commiphora mukul* patches.

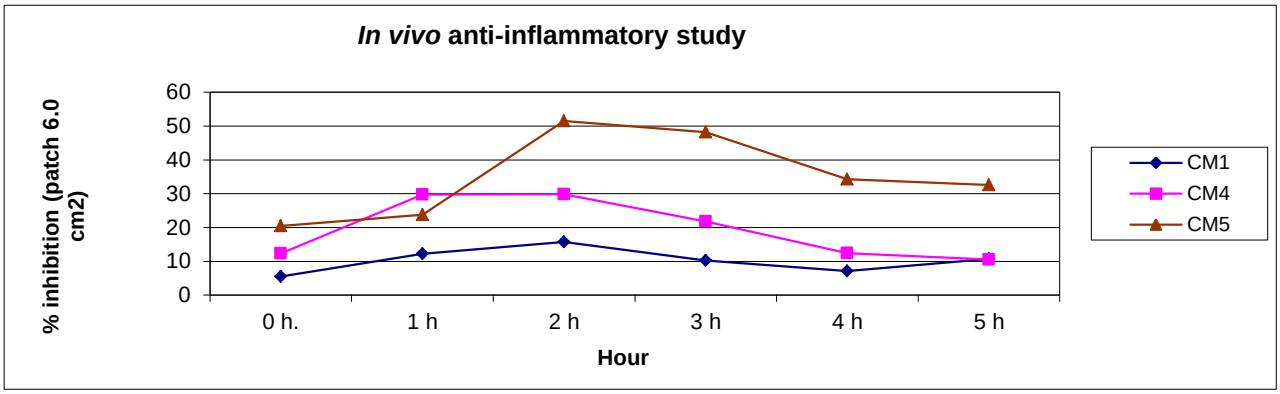


Figure 17: Comparative % inhibition of different formulation.

+++: Good; ++: Fair; + Poor

The results indicate that all formulation was stable with respect to physical properties up to 45 days. However extensive stability studies up to six months require confirming these results.

Table 15: Paw edema and % inhibition obtained on carrageenan challenge in male rats.

Formulation	0 Hour		1 Hour		2 Hour		3 Hour		4 Hour		5 Hour	
	Paw Vol.	% Inhibition	Paw Vol.	% Inhibition	Paw Vol.	% Inhibition	Paw Vol.	% Inhibition	Paw Vol.	% Inhibition	Paw Vol.	% Inhibition
Control	2.35 ± 0.21		2.76 ± 0.56		3.65 ± 0.47		3.61 ± 0.40		3.53 ± 0.23		3.69 ± 0.33	
CM1	2.22 ± 0.44	5.53	2.42 ± 0.24	12.21	3.07 ± 0.66	15.68	3.24 0.49	10.24	3.28 ± 0.14	7.14	3.29 ± 0.42	10.75
CM4	2.06 ± 0.15	12.34	1.94 ± 0.15	29.77	2.56 ± 0.08	29.86	2.82 ± 0.34	21.74	3.09 ± 0.30	12.42	3.36 ± 0.28	10.48
CM5	1.87 ± 0.12	20.42	2.15 ± 0.32	23.80	1.78 ± 0.37	51.23[a]	1.87 ± 0.69	48.19[a]	2.32 ± 0.65	34.32	2.49 ± 0.53	32.61

[a]p< 0.05 when compared with control (One way ANOVA followed by post hoc Scheffe's test). All values are expressed as Mean ± S.D. n=4

Conditions: Temperature/ Relative humidity- 40°C/ 75% RH.

Primary skin irritation study

The results of skin irritation studies showed negligible erythema with prepared films when compared with control. The absence of edema indicated that the polymeric patches are compatible with the skin and hence can be used for the transdermal application.

***In Vivo* study**

Carrageenan-induced rat paw edema has been considered as a useful model for studying the anti-inflammatory effect of profile. Table 15 and Figure 17, depicts comparison of mean paw volume and percentage inhibition of edema with duration after application of patches. The formulation CM5 was found to provide maximum protective effect as compared to CM1 and CM4.

It has been found that the formulation CM5 was shown to provide maximum protective effect as compared to CM1 and CM4. Maximum inhibition was observed in formulation CM1 and CM4 i.e. 29.86 and 15.68 respectively. This may be due of the less amount of drug release from these formulations. Burst release effect has been observed in all three formulations.

CONCLUSION

The highest % inhibition was found to 51.23% in CM5. Inflammation: A basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. Inflammation is now recognized as a type of nonspecific immune response. *Commiphora mukul*, is anti-inflammatory drug has been used in traditional Ayurvedic medicine for centuries in the treatment of arthritis. It has been associated with gastric upset as a major adverse effect and has to be administered 2-3 times a day. The transdermal patch delivery system may be an attractive choice of an alternative route of administration of this drug. Also presently no scientific reports are available on the formulation of transdermal drug delivery system of *Commiphora mukul.* Hence in this study an attempt has been made to prepare and evaluate the transdermal drug delivery system of *Commiphora mukul.* In this study, various transdermal matrixes patches containing *Commiphora mukul* of variable combination of ethyl cellulose /polyethylene glycol with enhancer (menthol: limonene) were prepared. It was desired to design a polymer matrix that allows one to control the release of drug by using most appropriate choice of polymeric ratio of ethyl cellulose and polyethylene glycol among prepared formulation. The prepared patches were studied with respect to physicochemical characters, drug-excipient interaction, dissolution, skin permeation, stability and *in vivo* anti-inflammatory studies. The combination of ethyl cellulose and polyethylene glycol produces smooth flexible films. The patches exhibited fair uniformity with respect to thickness and weight variation though little variation was observed in drug content. Moisture content and moisture uptake was found to decrease with an increase in polyethylene content. Dissolution and in *vitro* skin permeation studies revealed that the cumulative amount of drug permeated was decreased as the polyethylene content of the film increased. The film containing enhancer shows greater release as compared to film containing no enhancer. The drug release from all films was rapid in the initial hours and slower at the later hours. Zero order was found to be dominant mechanism for drug release from the films. Based on in *vitro* skin permeation studies, CM4 [PEG/EC, 1:5, menthol (36 µg): limonene (36µl)], was found to be better formulation as it released a maximum amount of drug. Variable result was obtained in *vivo* anti-inflammatory studies, as formulation CM5 [PEG/EC, 1:4, menthol (36 µg): limonene (36 µl)] was showing maximum protective effect on carrageenan induced rat paw edema. In stability studies, all the formulations were stable with respect to physical properties up to 45 days. In view of the overall result reported in the present study, it may be concluded that ethyl cellulose and polyethylene can be used in the design of a matrix type *Commiphora mukul* transdermal drug delivery system to prolog the release. Thus, based on above discussion, it can be justified that formulation CM5 has the best effective combination of polymer ethyl cellulose and polyethylene glycol, among the formulation studied. Still further study is required to formulate the patches with different enhancer that allows releasing more drugs across the skin.

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