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Formulation and *in- vitro* permeation studies of Ketoprofen-*Ficus reticulata* fruit mucilage transdermal patches

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ABSTRACT

The main objective of the present study was to develop matrix-moderated transdermal systems of Ketoprofen using various proportions of Ficus reticulata fruit mucilage. Physical evaluation was performed such as moisture content, moisture uptake, tensile strength, flatness and folding endurance. In-vitro penetration studies were performed in a Keshary-Chien diffusion cell. The matrix-type transdermal systems were prepared using Ketoprofen with Ficus reticulata fruit mucilage by the solvent evaporation technique. The interactions between Ketoprofen and Ficus reticulata fruit mucilage were performed. The transdermal patches were subjected to various physicochemical parameters viz., mechanical properties, permeation studies and skin irritation studies. The prepared patches possessed satisfactory pre-formulary and formulary characteristics. In-vitro permeation studies were performed using a Keshary-Chien diffusion cell across hairless Albino rat skin. Span 80, Glycerin and Propylene glycol in the formulation played a role as permeability enhancers. The patches were seemingly free of potentially hazardous skin irritation. The experimental results shows that the release of drug from the patch delayed in controlled manner as the proportion of Ficus reticulata increased. It was concluded that Ketoprofen can be developed as transdermal patches with Ficus reticulata fruit mucilage

Key words: Ketoprofen, *Ficus reticulata* fruit mucilage, transdermal patches, *in-vitro* permeation, skin irritation.

INTRODUCTION

Transdermal delivery has many advantages over conventional modes of drug administration, it thus avoids hepatic first pass metabolism and improves patient compliance. Intensive research has shown that transdermal route is a potential mode of delivery of lipophilic drugs in systemic circulation. Ketoprofen, a propionic acid derivative, is an NSAID. Ketoprofen is used in

musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis, and in peri-articular disorders such as bursitis and tendinitis. It is also used in dysmenorrhoea, postoperative pain, in painful and inflammatory conditions such as acute gout or soft-tissue disorders, and to reduce fever [1, 2]. The elimination half-life in plasma is about 3 to 4 hours [3]. The transdermal patches were evaluated *in-vitro* and for controlled release. Various experimental reports indicated that Ketoprofen as a good candidate for controlled release formulation. In this study, *Ficus reticulata* fruit mucilage was used as a matrix polymer for controlling release of Ketoprofen.

MATERIALS AND METHODS

Ketoprofen was obtained as a gift sample from Waksman Selman Pvt. Ltd, Anantapur, India. *Ficus reticulata* fruits were obtained from the main market of Anantapur and authenticated by the Botany department of Sri Krishnadevaraya University, Anantapur. Glycerin, Propylene glycol, Methyl paraben, Propyl paraben, Span-80 procured from S.D. Fine chemicals Mumbai. All the reagents used were of AR grade. The drug samples were characterized by UV spectrophotometric method. Solubility and pH were determined for their authentication.

Extraction of mucilage

The fresh ripen fruits of *Ficus reticulata* were obtained from main market of Anantapur, India. The fruits were thoroughly washed with water to remove dirt and debris then cut it into two pieces. The seeds which were present inside the fruit were removed. The pulps of the fruits were crushed and soaked in water for 5–6 hours, boiled for 30 min and left to stand for 1 hour to allow complete release of the mucilage into the water. The mucilage was extracted using a multi layer muslin cloth bag to remove the marc from the solution. Acetone (three times the volume of filtrate) was added to precipitate the mucilage [4]. The mucilage was separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in desiccator at 30°C and 45% relative humidity before use.

Purification of the Mucilage

The crude mucilage (1 %) was homogenized (Potter homogenizer) with cold dilute tri chloro acetic acid solution (5%). The solution was centrifuged (3500 rpm for 20 min), neutralized with sodium hydroxide by drop wise addition and then dialyzed for 30 hours against distilled water. The mucilage was precipitated with ethanol (in the quantities of three times the volumes) and washed successively with ethanol, acetone and diethyl ether.

Characterization of Mucilage:

The collected mucilage was evaluated for physicochemical characteristics viz., morphological characteristics, identification by chemical tests, Solubility, melting range, pH, Swelling index, Ash values, presence of foreign organic matter, test for lead and arsenic, Loss on drying, Density, compressibility index and angle of repose etc. The evaluation was carried out as per procedures described in official books.

Preparation of transdermal films

Various proportions of *Ficus reticulata* mucilage was taken in a beaker add Propylene glycol as plasticizer, Span-80 as penetration enhancer, Propyl paraben and Methyl paraben as preservatives and finally Ketoprofen(100 mg) were added (Table 1) with continuous stirring using magnetic stirrer for 30 min at 500 rpm. The above mixture was poured within the glass bangles (6.1 cm diameter) placed on mercury surface in a Petri dish. The rate of evaporation was

controlled by inverting a funnel over the Petri dish. After 24 hours the dried films were taken out and stored in desiccator [5, 6].

Table 1: Physicochemical characterization of *Ficus reticulata* mucilage

Properties	***Ficus reticulata***
% yield (g /kg)	125±10.567
Solubility	Soluble and forms colloidal solution, in Luke warm water. Practically insoluble in ethanol, acetone, ether and chloroform.
Odour	Characteristic.
Appearance	Lustrous.
IDENTIFICATION	
a) Mounted in 96% ethanol	Transparent angular masses
b) Mounted in ruthenium red	Particles stained red.
c) Mounted in Iodine solution	Particles stained blue
Ave. particle size (µm)	212.58±20.515
Weight loss on drying (%)	6.33±5.210
Acid insoluble ash (%)	1
Swelling Index	85±11.246
pH	7.0
Test for Carbohydrate (Mollish test)	+
Test for Tannins (Ferric chloride test)	-
Test for chloride (Silver-nitrate test)	-
Test for Sulphate (Barium chloride test)	-
Uronic acid test	+
Test for foreign matter (%)	NMT 0.1
Test for heavy metal (lead).	20-25 ppm
Test for Arsenic.	<1 ppm
Solubility	Soluble in lukewarm water, Practically insoluble in ethanol, acetone, ether and chloroform
Charring (^{0}C)	Decomposes above 200
Density of liquid (0.5% w/v)	1.368±0.099
Microbial count (cfu/g)	Bacteria:15; Fungi: 7
Angle of repose (θ°)	27.83±1.84
Loose Bulk density (g/cm^3)	0.58±0.04
Tapped bulk density(g/cm^3)	0.79±0.05
Carr's Index	26.58±1.29
Hausner's ratio	1.25±0.06
Viscosity of mucilage(mPas)	
0.1%	3.26±0.29
0.2%	5.69±0.49
0.3%	7.89±0.12
0.4%	9.94±0.65
0.5%	12.67±0.37
Number of experiments (n) = 5; + Present. – Absent; ppm= Parts per million	

Compatibility Studies:

Differential Scanning Calorimetry:

Differential Scanning Calorimetry (DSC) curves were obtained by a differential scanning calorimeter (Schimadzu DSC-50, Tokyo, Japan) at a heating rate of 10°C/min from 25°-250°C in nitrogen atmosphere (20 ml/min) with a sample weight of 3mg.

Fourier Transform Infra-Red (FT-IR) spectral analysis:

Fourier–Transformed Infrared (FT–IR) spectrums of Ketoprofen with *Ficus reticulata* fruit mucilage were obtained individually and in combinations on a Fourier-Transform Infrared (FT-IR) spectrophotometer, (Perkin Elmer, spectrum-100, Japan using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm^{-1} and the resolution was 1 cm^{-1}. This spectral analysis was employed to check the compatibility of drugs with the mucilage used. The IR spectrum of pure drug and the formulations were shown in fig.1 and 2.

Fig.1. FTIR spectrum of Ketoprofen pure drug

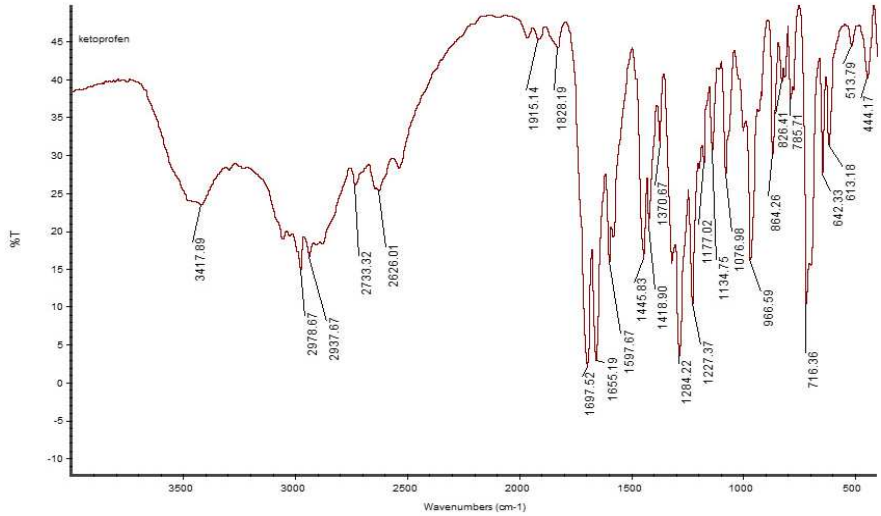
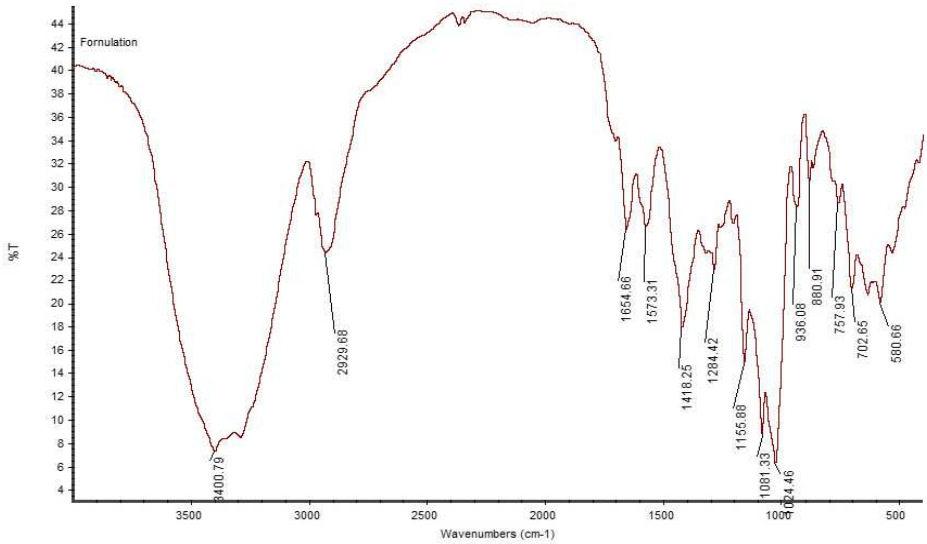


Fig.2. FTIR spectrum of formulated transdermal patches



Evaluation of Physicochemical parameters

Thickness:

The thickness of the patch was determined using Digital caliper (BAKER-EC 10, Hyderabad, India). The mean thickness was measured at five different points of the film.

Determination of tensile strength:
Tensile strength was determined by using computerized Precisa bottom-loading balance, with necessary modifications. A 1 X 1cm patch was taken and subjected to studies.

Flatness and elongation brake:
Longitudinal strips were cut out from the prepared transdermal patches. The flatness was determined at various points by using vernier calipers [7]. The percentage elongation brake was determined by noting the length just before the break point and substituted in the eq.1.

Elongation (%) = $L_1 - L_2$ X 100/ L_2 (1)

Where
L_1 = final length of each strip
L_2 = initial length of each strip.

Folding endurance:
Folding endurance of patches was determined by repeatedly folding a small strip of film (2 X 2 cm) at the same place till it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value [8].

Moisture content:
The strips were then weighed individually and kept in a desiccator containing activated silica at 30°C for 12 hours. The films were reweighed individually until a constant weight was obtained [9]. Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film. The prepared patches were cut into 20 × 50 mm strips. The film was weighed and kept in a desiccator containing calcium chloride at 30°C and dried for at least 12 hours. The film was weighed until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight.

Moisture uptake:
The physicochemical studies like moisture content and moisture uptake provide the information regarding the stability of the formulation. The moisture content was determined by keeping the drug matrix patches in a desiccator containing activated silica until they showed constant weight. The percentage moisture content was calculated from the weight differences relative to the final weight. The water absorption capacities of various films were determined at 75% and 93% relative humidity (RH). Films were cut into 25 × 60 mm strips. A strip was weighed and kept in a desiccator at 40°C for 24 hours, removed and exposed to RH conditions of 75% (containing saturated solution of sodium chloride) and 93% (containing saturated solution of ammonium hydrogen phosphate) in different desiccators at room temperature. Then the films were measured periodically to constant weights. The water absorption capacity of the films (in weight %) was calculated in terms of percentage increase in the weight of film over the initial weight of the specimen.

Drug content determination of film:
Four pieces of 1 cm^2 each (1 X 1 cm) were cut from different parts of the prepared transdermal patch. Each was taken in separate stoppered conical flasks containing 100 ml of suitable dissolution medium (0.1N HCL: CH_3OH mixture) and stirred vigorously for 6 h using magnetic stirrer. The above solutions were filtered and suitable dilutions were made. Absorbance was observed using UV-Visible double beam spectrophotometer (Elico SL 210, Hyderabad, India) at

their respective wavelengths, against a blank solution which was prepared by the same protocol but not containing drug.

***In-Vitro* skin permeation studies:**

The *in-vitro* skin permeation of drug from formulated patches was studied using rat dorsal skin. The skin was cleared from epidermal hair and any adhering subcutaneous tissue and blood vessels were removed. The skin was mounted overnight (12 hours) on receptor side to remove any water-soluble (UV absorbing) material. The *in-vitro* skin permeation of Ketoprofen from formulated transdermal patches was studied using Keshary-Chien diffusion cell. The upper part of diffusion cell consists of the donor compartment which contains the active ingredient and the carrier adhesive/patch; the bottom part contains the receptor solution, the temperature was controlled by using water jacket and sampling through the sampling port.

Fig.3. Zero order plots of penetration profiles of formulated transdermal patches

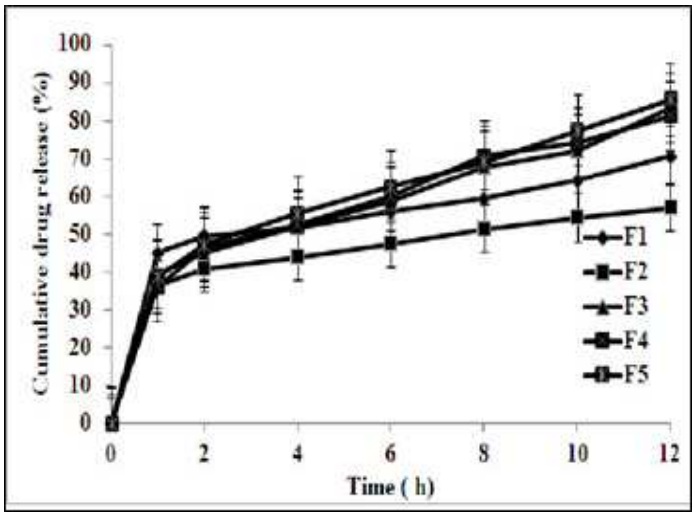
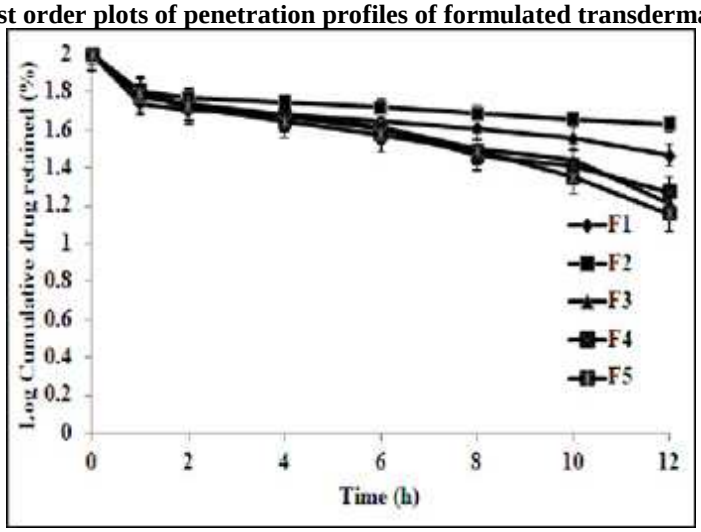


Fig.4. First order plots of penetration profiles of formulated transdermal patches



The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm^2 and 17.5 ml respectively. The temperature was maintained at 37±2°C. The receptor compartment contained 17.5 ml of phosphate buffer saline (PBS) IP (pH 7.4) stirred by magnetic stirrer. At predetermined time intervals the samples (1.0 ml) were withdrawn and replaced with the same volume of fresh receptor solution till 48 hours. Absorbance of the samples was measured at 254

nm. The experiments were done in triplicates, simultaneously blanks were also performed. The kinetic plots of the obtained permeation data were plotted in fig. 3 to 7.

Fig.5. Higuchi's plots of penetration profiles of formulated transdermal patches

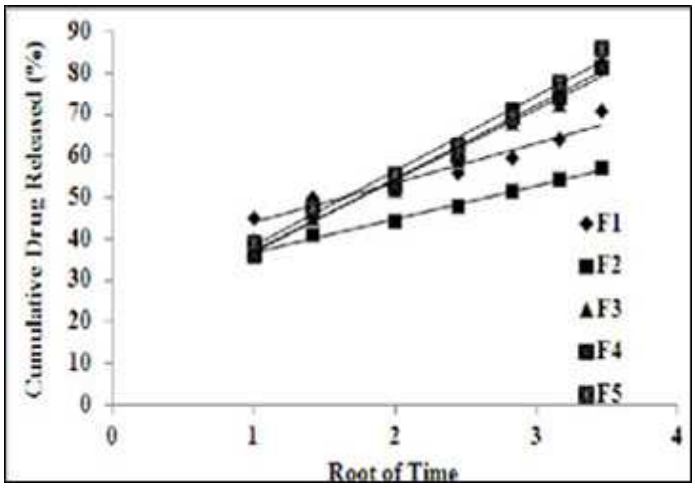
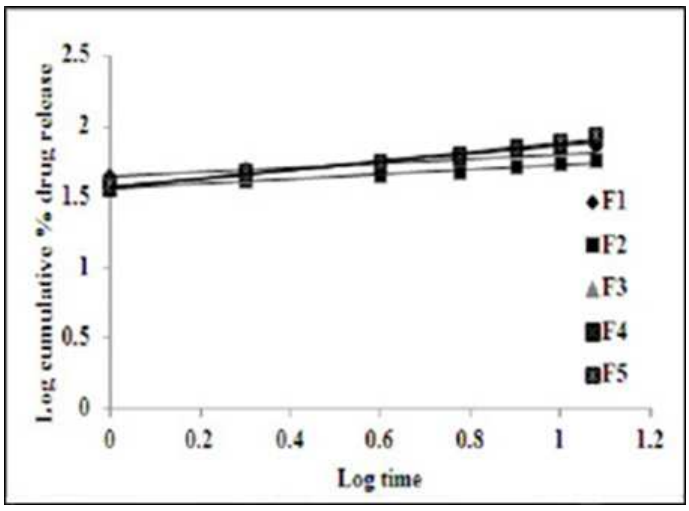


Fig.6. Korsmeyer Peppa's plots of penetration profiles of formulated transdermal patches



Evaluation of skin irritation potential:

The skin irritation studies were carried out using modified Draize test [10]. The hair at dorsal area of rabbits were removed 24 hours before test, one side of the back of each rabbit i.e. untreated skin area serves as the control for the test. Medicated patch was secured on experimental side using adhesive tape and the non-medicated patch was adhered on the control side of six rabbits [11, 12]. These attached patches were covered with waterproof covering to approximate the condition of use. The medicated patches were changed after 48 hours and the fresh patches were secured at the same site. The patches on the control side were not changed. The patches were secured on the back for seven days. After removal of patch after a week each of the areas were examined for any sign of erythema or edema. These results were shown in table 5.

Fig.7. Hixson Crowell's plots of penetration profiles of formulated transdermal patches

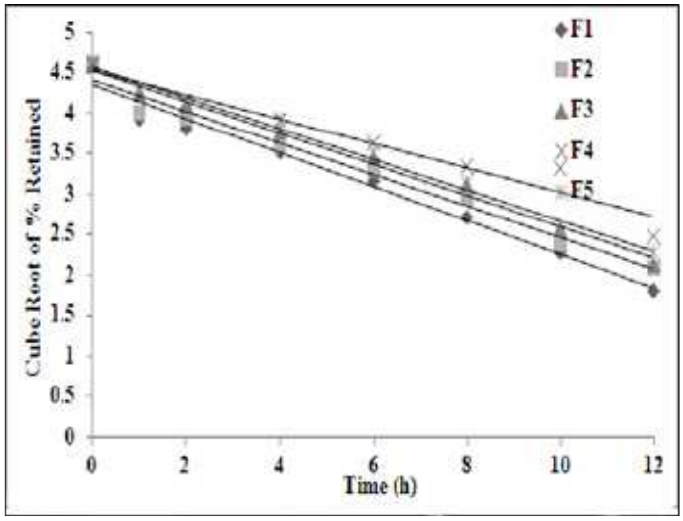


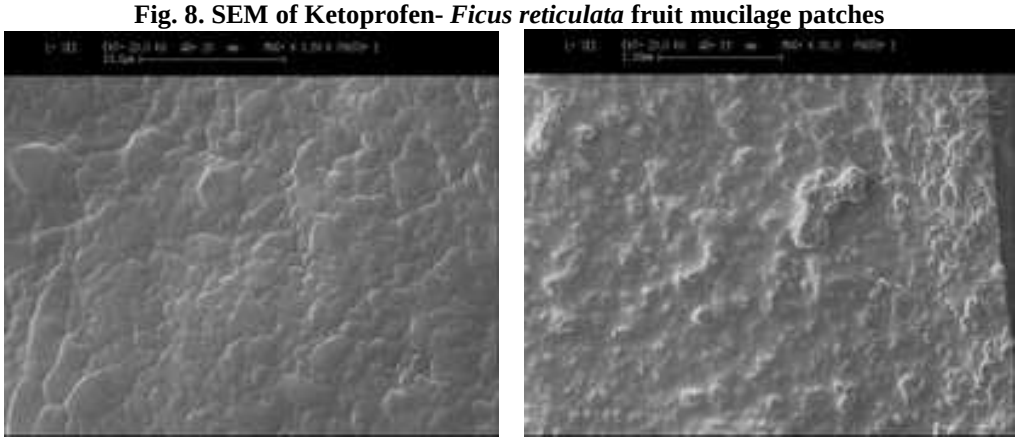
Table 5: Results of skin irritation test of formulated transdermal patches

Formulation	Visual observation	
	Erythema	**Edema**
Normal	0.00±0.00	0.00±0.00
Adhesive tape(USP)	1.31±0.21	1.60±0.25
F5 (Ketoprofen-patch)	1.52±0.35	1.24±0.17
Blank	1.51±0.14	1.18±0.42
Formalin (0.8% v/v)	3.75±0.18	3.39±0.36
Visual observation values are expressed as Mean ±SEM, n=6; ** Significant compared to formalin ($p<0.05$);* *F5=Ketoprofen Ficus reticulata fruit mucilage patch;* *Blank= Patch without drug*		

Scanning Electron Microscopy (SEM) studies:

The Scanning Electron Microscopy (MERLIN Field Emission Scanning Electron Microscope (FE-SEM), Carl Zeiss, Germany) of the selected transdermal patches. The SEM photographs were shown in fig.8.

Fig. 8. SEM of Ketoprofen- *Ficus reticulata* fruit mucilage patches



Stability studies:
Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS samples at 40± 0.5 °C and 75 ± 5% RH for 3 months [13].

RESULTS AND DISCUSSION

The thicknesses of formulated matrix transdermal patches were ranged from 630±35.6 to 690±25.6 µm. The Tensile strength of formulated patches was ranges from 0.285 ± 0.25 to 0.326 ± 0.10 kg/cm^2 (Table 3). The elongation of formulated matrix transdermal patches were ranged from 15.33±0.89 to 26.23±0.84 (N/mm^2). The folding endurance of formulated patches was ranged from 98±1.8 to 124±0.9 (Table 3). The moisture content was ranged from 2.645±0.35 to 2.854±0.56% (Table 4). The drug content in formulated films was ranged from 97.4± 0.02 to 100.7± 0.45%. The patches did not show any visible erythema or edema with the formulation or the base used. In the present work stability study was carried out for selected formulation (F5) at 40± 0.5°C and 75±5% RH for 3 months using programmable environmental test chamber (Remi, India).

Table 2: Different formulae of transdermal patches

Ingredients	F1	F2	F3	F4	F5
Ketoprofen (mg)	100	100	100	100	100
Ficus reticulata fruit mucilage (%)	5	10	15	20	25
Glycerin(ml)	0.3	0.3	0.3	0.3	0.3
Propylene Glycol(ml)	0.18	0.18	0.18	0.18	0.18
Span-80 (ml)	0.06	0.06	0.06	0.06	0.06
Methyl paraben(g)	0.025	0.025	0.025	0.025	0.025
Propyl paraben(g)	0.015	0.015	0.015	0.015	0.015
Water up to (ml)	20	20	20	20	20

Table 3: Result of mechanical properties of formulated transdermal patches

Parameter	Thickness (µm)	Tensile strength (N/mm^2)	Elongation (%)	Folding endurance
F1	630±35.6	0.294 ± 0.14	15.33±0.89	98± 1.8
F2	650±62.5	0.285 ± 0.25	18.22±0.23	124±0.9
F3	685±55.8	0.311 ± 0.05	22.66±0.36	115±1.2
F4	690±25.6	0.325 ± 0.12	24.95±0.39	99±1.5
F5	635±29.6	0.326 ± 0.10	26.23±0.84	119±1.4
Number of trials (n) = 3				

Table 4: Result of mean weights, moisture content, moisture uptake and dug content of formulated transdermal patches

Formulation	Weights (g)	Moisture content (%)	Moisture uptake (%)		Drug Content (%)
			RH 75%	RH 93%	
F1	1.561±0.51	2.848±0.12	3.206± 0.37	6.145 ± 0.01	97.4± 0.02
F2	1.584±0.12	2.851±0.23	4.125± 0.52	5.249 ± 0.12	98.3± 0.19
F3	1.564±0.14	2.645±0.35	3.130± 0.73	3.936 ± 0.49	99.7± 0.23
F4	1.566±0.34	2.758±0.35	2.210± 0.96	5.219 ± 0.20	100.2± 0.22
F5	1.597±0.01	2.854±0.56	3.206± 0.37	3.906 ± 0.59	100.7± 0.45
Number of trials (n) = 3					

The prepared patches did not show any signs of cracking, which might be attributed to the addition of the plasticizer, Propylene glycol. The folding endurance measures the ability of patch to withstand rupture. The folding endurance was measured manually and results indicated that the patches would not break and would maintain their integrity with general skin folding when used. The moisture content of the prepared transdermal film was low, which could help the formulations remain stable and from being a completely dried and reduce brittleness during storage. The patches did not show any visible erythema or edema with the formulation or the base used. After the accelerated stability studies the patches were evaluated for physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content and moisture uptake, drug content as well as drug release. The absence of edema indicates that the polymeric patches are compatible with the skin and hence can be used for transdermal application. The drug permeation from prepared patches was sustained within the therapeutic range. The stability study indicates that the formulation is quite stable at accelerated conditions.

CONCLUSION

This investigation revealed that *Ficus reticulata* fruit mucilage appears to be suitable for use as a matrix former in the manufacturing of transdermal patches because of its satisfactory physical and mechanical properties. The *In-vitro* permeation data revealed that dried *Ficus reticulata* fruit mucilage can be used as a matrix former in transdermal delivery systems.

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