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# Formulation and evaluation of transdermal patches of isolated compound from plant extract for in-vivo anti-inflammatory activity

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## ABSTRACT

The aim of present research work was to formulate and evaluate the transdermal patches of isolated compound from plant extract for their *in-vivo* anti-inflammatory activity. Suitable parameters for the phytochemical screening, physicochemical characterization, preformulation of transdermal patches, drug content, *in-vitro* drug release and *in-vivo* anti-inflammatory activity of formulated transdermal patches. The anti-inflammatory activity decreased with increasing time in case of control (Indomethacine) group whereas the curcumin (400 mg/kg) showed maximum activity at maximum time (12 h) the activity increasing with increasing time intervals. The activity of formulation having dose rate 20 mg/kg bodyweight was decreasing with time intervals. The formulation having dose rate 40 mg/kg was better than formulation 20 mg/kg. Curmumin showed maximum activity compared to other formulations and standard. These parameters can serve as quality characters and criteria for the evaluation of the identity and authenticity of the plant.

**Keywords:** Transdarmel, Extract, Anti-inflammatory, Curcumin,Indomethacine

## INTRODUCTION

Herbal medicine also referred to as botanical medicine or phytomedicine refers to the use of plant seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Herbal medicines are gaining popularity worldwide for human well-being and healthcares based on their positive therapeutic results and are gaining resurgence among the consumers throughout the world (Ajayi *et al.,* 2011; Das, 1991; Srikanthmurty, 2000). The Consumption of herbal medicines is widespread and increasing. There is growing concern about diminishing populations of some medicinally important plant species, loss of genetic diversity, local extinctions and their habitat degradation. Herbal revival is happening all over the planet. People are taking note of herbal remedies for the treatment of various kinds of diseases in place of conventional allopathic treatment. There is increasing attention in traditional system of medicine due to superior outcome and minimum side effect as compare to conventional medicines. There are main three reasons for popularity of herbal drugs:

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1. Modern drugs are failing to efficiently treat many of the health conditions like gout, arthritis, tuberculosis, skin diseases, AIDS, upper and lower respiratory problems etc.
2. Trustworthy and safe
3. They produce better results without side effects

Herbal remedies are commonly used to treat various health conditions like tuberculosis, skin diseases (psoriasis), melanoma, hepatic, immune-deficiency, acute respiratory and rheumatologic disorders. World health organization (Geneva) estimates that about 33.33 percentage of the world's population now a day use herb and formulations made up from natural sources for to treat various ailments(Varrier, 1996; Jagtap &Karkera, 1999; Ahmad *et al.,* 1998).The easiest approach for administration of drugs and bioactive molecules is through the transdermal route. Many of the therapeutic agents undergo drug-food interaction when it is given by oral route. However, transdermal route avoids the wastage of the drug and provides an attractive way of usage (Sato *et al.,* 1997). A wide range of medications and chemical entities are available in the market as transdermal dosage forms. The delivery of drugs via the transdermal route has certain merits such as accurate dosing, termination of medication, easy to apply (self-medication) and avoids sensation of nausea which is observed when the drug is administered through oral route (Purseglove *et al.,* 1981).

The concept of transdermal drug delivery system (TDDS)was proposed by United States (US) scientists in and around 1980s and 90s. The first TDDS was introduced in the US over 20 years ago. Initially a number of the US pharmaceutical companies showed interest in developing the transdermal dosage forms. But excitement dwindled to disappointment, because of the limitation that only few drug molecules could be designed for transdermal delivery (Ashraf *et al.,* 2012; Priyadarsini, 2014).

## MATERIALS & METHODS

### Chemicals

Curcumin, Chitosan, Poly vinyl alcohol, Poly ethylene glycol, Glycerol, Lactic acid and Carrageenan. All the chemicals were purchased from S D fine chemicals Mumbai and all chemicals and materials were of analytical grade used for this project work.

Selection and Authentication of Plant

The rhizomes of *Curcuma longa* were collected from Bhopal and authenticated by botanist from the Department of Botany, Saifia Science College, and Bhopal. Plant authentication number:(specimen number BT/10/22/Saifia/Science/College/Bhopal)

### Solvent Extraction

Collected plant material washed under running tap water and kept in shade for drying. Plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of rhizomes of *Curcuma longa* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. Dried extract was weighed and percentage yield for each extract was determined (Maiti *et al.,*2007).

### Phytochemical investigation:

Qualitative photochemical investigation: The phytochemical investigation was carried out by procedure given byKokate*et al.,* 1993.

### Isolation of Plant Component

### Thin layer chromatography

Thin Layer Chromatography of *Curcuma longa* extract was carried out on silica gel coated and activated (0.4-0.5mm thick) glass plates in an oven at 100°C for 30 min. These plates were developed in an organic solvent mixture, air dried and visualized under UV light. Spots were observed with distinct RF value (Rana *et al.,* 2016).

$R_f$= Distance travelled by the compound.
Distance travelled by the solvent front.

### Column chromatography

For the isolation of bio active components from methanol extract of *Curcuma longa* columns was prepared. The bottom of the columns (45 cm length, 12 cm diameter) were packed with adsorbent cotton, above which silica gel (60 g) was filled as a slurry in petroleum ether. Care was taken to prevent entrapment of air bubbles into the columns. About 2 g of the methanol extract of *Curcuma longa* was dissolved in methanol, dispersed uniformly in 20 g of silica gel and loaded over the filled columns. The column contents were eluted with gradient elution starting with (100:0) pet ether followed by pet ether, chloroform, ethyl acetate and methanol. Each time, 30 ml of elute was collected. The separation was done by gradient elution with low polar to high polar (gradient from 100% low polar/0% high polar to 0% low polar/100% high polar) using the flow rate of 1 ml/min. Elution of different components was monitored by TLC to know the nature of compounds in the fractions and homogeneity of the compounds. Fractions showing similar spots with same Rf values were pooled and concentrated with evaporating temperature of 40°C (Vavilova, 1990).

### Preformulationstudies

Preformulation studies were conducted to confirm the drug identity, solubility profile for drug release receptor media and for compatibility of drug with other excipients of matrix transdermal formulations. In our experiment, Preformulation studies were performed or classified into three sections:

A. Preparation of phosphate buffer saline, pH 7.4
B. Preparation of standardcurve.
C. To acquired physicochemicalproperties.

### A. Preparation of phosphate buffer saline, pH7.4:

Phosphate buffer saline pH 7.4 was prepared according to Indian pharmacopeia (IP, 2010). 8.0 g of sodium chloride (NaCl), 0.19 g of potassium dihydrogen phosphate ($KH_2PO_4$) and 2.38 g of disodium hydrogen phosphate ($Na_2HPO_4$) was dissolved in sufficient water ($H_2O$) and volume was made up to 1000ml with distilled water. The buffer pH was arranged to 7.4 using hydrochloric acid (HCl) and sodium hydroxide(NaOH).

### B. Preparation of standardcurve:

Standard curve of curcumin was prepared in ethanol by using UV spectrophotometric analysis.

Stock solution A: Stock solution A was prepared by dissolving 10mg curcumin in ethanol and volume make up to 10ml with ethanol to produce 1000µg/ml solution.

Sub-stock solution B: 2.5 ml of stock solution A was taken into 25 ml volumetric flask; make up volume up to 25ml to produce sub-stock solution B(100µg/ml).

**Preparation of aliquots:** Further, serial aliquots range from 1 µg/ml to 5 µg/ml was prepared from sub-stock solution B.

Scanning of aliquots was carried out spectrophotometrically via Shimadzu 1700 UV-Visible spectrophotometer ranging from 300 nm to 500 nm. The λmax of curcumin was obtained at wavelength of 424 nm. Calibration curve of curcumin was prepared by plotting absorbance value against concentration of drug.

**C. Investigation of physiochemical characteristics of curcumin**

**Organoleptic characteristics:**

**Color:** Color of curcumin was determined by physical appearance.

**Taste and odor :** Extremely minute amount of curcumin was used to acquire taste of drug with the help of tongue as well as smelled to get the odor of drug.

**Melting point:** A small amount of pure curcumin was taken in fused capillary tube. Fused tube positioned in melting point apparatus (VEEGO).

**Determination of partition coefficient:** Partition coefficient is one of the most important parameter for a measurement of lipophilicity of drugs, which can help to predict its potentiality to cross the biological membrane. The partition coefficient (Po/w) of drug was calculated by using the following formula: Po/w = Concentration of drug in n-octanol / Concentration of drug in PBS (Afaq *et al.,* 2002).

**Development of transdermal patch:**

Preparation of transdermal patch involves two steps:

1. Preparation of backing membrane:
2. Preparation of matrix system:

**Preparation of backing membrane:**

A backing membrane of transdermal patch was prepared from 4%w/v aqueous solution of PVA (poly vinyl alcohol). The mixture (4g PVA in 100 mL water) was stirred with heating between 70-80 °C till a clear solution was obtained. Obtain clear solution was cooled at room temperature and sonicated for 15 min to remove any entrapped air. 10 ml of this solution was poured into a petri dish (25 $cm^2$ area) and solution was dried for 24 hours at room temperature.

**Preparation of matrix system**

The matrix type patches of curcumin were formulated using different quantity of chitosan and different permeation enhancers by solvent evaporation method. Polymer solution was prepared by taking the appropriate quantity of chitosan and dissolved in 4% v/v aqueous solution of lactic acid in 50 mL. The chitosan solution was homogenized at 1500rpm until clear solution obtained (Approx. 2-3 h). Glycerol used as plasticizer were added and homogenized for 30 min. Drug (50 mg) was added and 3-4 mL methanol (Work as co-solvent for drug) added into homogeneous dispersion and homogenized for 30 min. A known quantity of permeation enhancers (Propylene glycol or oleic acid) were added drop by drop into the solution and again homogenized for 30 min. To remove entrapped air bubble in the dispersion, the beaker was placed in the sonicator at room temperature until all the air bubbles were removed. The uniform dispersion was poured on the PVA backing membrane cast earlier. The petri dish was covered with an inverted funnel to control the rate of evaporation of solvent. This matrix dispersion was kept undisturbed at room temperature for 48 hours in order to evaporate the solvent. After complete drying, the transdermal patches were peeled from the Petri dish and cut in square patches. The patches were properly wrapped in aluminium foil and kept in desiccators for further uses (Ammon & Wahl, 1991).

Table 1: Composition of prepared transdermalpatches

| S. No. | Formulation Code | Chitosan (mg) | Drug (mg) | Glycerol (mL) (mL) | Propylene glycol | 4% Aqueous solution of lactic acid (mL) |
|---|---|---|---|---|---|---|
| 1. | FC1 | 200 | 50 | 0.15 | 0.25 | 15 |
| 2. | FC2 | 300 | 50 | 0.15 | 0.25 | 15 |
| 3. | FC3 | 400 | 50 | 0.15 | 0.25 | 15 |
| 4. | FC4 | 500 | 50 | 0.15 | 0.25 | 15 |
| 5. | FC5 | 600 | 50 | 0.15 | 0.25 | 15 |

**Evaluation of Prepared transdermal patches**

**Physical Appearance**

All the prepared patches were visually inspected for physical appearance like clarity, smoothness, color, flexibility.

**Thickness**

The thickness of the prepared patches was measured by using digital Vernier caliper

(Aerospace, range 0-150 mm) at three random sites.

**Weight variation**

The three patches of each formulation were cut in 3.5×3.5 $cm^2$ and weighed in digital balance (Contech Analytical Balance) individually.

**Folding endurance**

This test is very important for determining and checking the efficiency of plasticizer. Folding endurance is defined as the number of times polymeric film folds without break and crack. This test was carried out manually by repeatedly folding a patch at same location until it broke.

**Percentage Moisture Loss**

Percentage moisture loss parameter was used to check the physical stability of patches in dry condition. The three patches (1.5×1.5 $cm^2$) from each formulation were individually weighed accurately and placed in a desiccators having anhydrous calcium chloride at room temperature (26±1 °C) for 72 hr. During this interval, the patches were weighed again after 12, 24 and 48 hr. The individually percentage moisture loss was calculated from the formula given below:

$$\% \text{ Moisture loss} = \frac{\text{Initial Mass - Final Mass}}{\text{Initial Mass}} \times 100$$

**Water vapor permeation test**

The quantity of moisture transmitted through unit area of patch in unit time was determined by water vapor permeation test. Glass vials of equal volume were used in this study. Glass vials were washed properly and dried in hot air oven. 1 gm anhydrous calcium chloride was taken in each glass vial and the medicated films were fixed over the brim of the glass vials with the help of adhesive tape. Then these glass vials were weighed accurately and stored at 84% RH in desiccators having saturated solution of potassium chloride. These glass vials were removed (from desiccator) and reweighed after 24, 48, 72 hrs. To determine the weight gain. Water vapor permeation (WVP) was calculated by using following formula:

$$WVP = \frac{WT}{S}$$

Where, W is weight of water vapor permeated, T is the thickness patch and S is Surface area exposed by film over the brim of vials in square centimeter.

Weight of water vapor permeated (W) was determined as follow:

W= Final weight – Initial weight (After different time interval)

**Determination of drug content**

Films of 3.5×3.5 $cm^2$ were cut and placing in a volumetric flask containing 100 ml of phosphate buffer saline and ethanol, at room temperature (26±1 °C). The mixture was stirred at magnetic stirrer for 24 h and solution was filtered through Whatman filter paper (pore size 0.45µm). Absorbance of filtrate was taken by UV visible spectrophotometer at 425 nm. The drug content was calculated from the absorbance value (Jain *et al.,* 2019).

***In-Vitro* drug release studies**

*In-vitro* release studies were carried out by modified Franz diffusion cell. The release studies were performed using egg membrane (Biological membrane). The egg membrane was removed using diluted solution of hydrochloric acid. Subsequently egg membrane was cleaned using distilled water, and immersed in phosphate buffer saline and ethanol for half an hour. A section of egg membrane was cut and tied on donor compartment using thread. The transdermal patch was placed on donor compartment in such manner facing the matrix side of the patch to the egg membrane and backing membrane opposite side. The receptor compartment of modified diffusion cell was filled with adequate volume of phosphate buffer saline (pH 7.4) and ethanol, whose temperature was kept 37±2 °C and stirred at 500 rpm. Followed by the holder (Donor compartment) containing membrane and patch was placed on receptor compartment of modified diffusion cell. Whole system was positioned on a magnetic stirrer and solution of receptor compartment was stirred continuously at 500 rpm using a magnetic bead. The release study was carried out for 48 hours (Jain *et al.,* 2012).

The samples (3.0 ml each time) were withdrawn from receptor compartment at different time intervals and added an equal volume of phosphate buffer saline (pH 7.4) and ethanol to maintain sink condition. The collected samples were subjected to UV spectrophotometer at 425 nm against phosphate buffer saline (pH 7.4) and ethanol as blank.

**Animal studies**

The animal studies were carried out according to approval of institutional animal ethical committee (Protocol No: PH/IAEC/VNS2K19/11). Wistar rats were housed in cages, given free excess of laboratory diet and water. All rats were housed in laboratory having facilities of air conditioner and cycle of 12 h dark and 12 h light.

**Skin irritation study**

Young rats having weight 200-250 g were divided into two groups, each containing three rats. The backside of rats was shaved before 12 h to conduct the experiments. To the control group (group I), an adhesive tape was applied. To the test group (groups

II), transdermal patches FP3 was applied on the dorsal surface of the rats. The animals received new patch every day up to 5 days. The patch was detached after 5 days. The rats were observed for erythema and edema sign after study period of 5 days and scored by draize scoring method as score 0 given for no erythema, 1 given for very slight erythema (light pink), 2 given for well- defined erythema (dark pink), 3 given for moderate to severe erythema (light red), and 4 given for severe erythema (dark red).

**Therapeutic study (Carrageenan induced paw edema)**

Young rats having weight 200-250 g were used to perform the experiment. The backside of rats was shaved 12 h before starting the experiments. The study was divided into two sections; section 1 in which formulations were applied before 2 hr of carrageenan insult and section 2 in which formulations were applied before 12 hr of carrageenan insertion.

Section 1 has two groups and each group contain 3 rats. Section 1 was utilized for determination of immediate drug release and action. Group I (Vehicle treated+0.2 ml of 1% carrageenan sub-plantar) rats have received only a sub-plantar injection of carrageenan. Group II (Transdermal patch of curcumin + 0.2 ml of 1% carrageenan sub-plantar) have received prepared patch. Similarly section 2 has also two groups, To induce paw edema in section 1 rats, after 2 h of application of formulations in all the rats were injected (sub-plantar) with 0.1 mL of a 1% w/v homogeneous suspension of carrageenan (in double distilled water) in the right paws.

Whereas in section 2, carrageenan injection was injected in rats after 12 h of application of formulation. The hind paw volume was measured (In both of section) at 0, 1, 2, 3, 4, 5, 6 and 24 h after carrageenan injection using a plethysmometer and expressed as a percentage of edema relative to initial hind paw volume.

***In vivo* Anti-Inflammatory Activity**

**Carrageenan-Induced Rat Paw Edema Model**

The rats were divided into five groups containing six rats each and acute inflammation was induced according to edema assay. The extract and formulations were evaluated for the anti-inflammatory activity. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% Carrageenan in normal saline in the right hind paw of the rats. After 30 min of carrageenan induction standard marketed preparation was applied to animals of group II whereas group III, IV and V animals received topical application of curcumin extract alone and test formulations (10 and 20 mg/kg) respectively. Reduction in edema was measured at every 15 min up to 12 h. Percent change in paw volume was calculated. The anti-inflammatory activity was calculated as percentage inhibition of Carrageenan induced paw edema using the following formula.

% Inhibition = [(A control - A test)/ A control] x 100

**Statistical Analysis**

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Bonferroni test. Differences were considered as statistically significant at $P < 0.05$, $P<0.001$ when compared with control.

## RESULTS & DISCUSSION

**Percentage Yield**

The rhizomes of the *Curcuma longa* were extracted by Soxhlet extraction and the percentage yield calculated by the formula Yield (%) = Weight of the residue obtained/Weight of the plant material taken×100 was found to be % (petroleum ether) and % (methanol).

Table 2: Percentage yield of different fruits and rhizomes extracts of Curcuma longa

| Plant Name | Percentage yield (%) | |
|---|---|---|
| | Pet. ether | Methanol |
| Curcuma longa | 1.21 | 2.72 |

**Phytochemical Analysis**

Qualitative phytochemical testing of extracts was done to study the presence or absence of various phytochemical constituents using standard tests (Kokate *et al.*, 1993). The results of Curcuma longaare shown in table.

Table 3: Phytochemical evaluations of different extracts of *Curcuma longa*

| S. No. | Experiment | Results | |
|---|---|---|---|
| | | Pet Ether Extract | Methanolic extract |
| | Test for Carbohydrates | | |
| 1 | Molisch's Test | -ve | -ve |
| 2 | Fehling's Test | -ve | -ve |
| 3 | Benedict's Test | -ve | -ve |
| | Test for Protein & Amino acids | | |
| 4 | Biuret's Test | -ve | -ve |
| 5 | Ninhydrin Test | -ve | -ve |
| | Test for Glycosides | | |
| 6 | BorntragerTest | -ve | +ve |
| 7 | Killer killaniTest | -ve | +ve |
| | Test for Alkaloids | | |
| 8 | Mayer's Test | -ve | +ve |
| 9 | Hager's Test | -ve | +ve |
| 10 | Wagner's Test | -ve | +ve |
| | Test for Saponins | | |
| 11 | Froth Test | +ve | +ve |
| | Test for Flavonoids | | |
| 12 | Lead acetate | -ve | +ve |
| 13 | Alkaline reagent test | -ve | +ve |
| | Test for Triterpenoids and Steroids | | |
| 14 | Libermann-Burchard Test | -ve | +ve |
| 15 | Salkowski Test | -ve | +ve |
| | Test for Tannin and Phenolic Compounds | | |
| 16 | Ferric Chloride Test | -ve | +ve |
| 17 | Gelatin Test | +ve | +ve |
| 18 | Lead Acetate Test | +ve | +ve |

Isolation of Components:

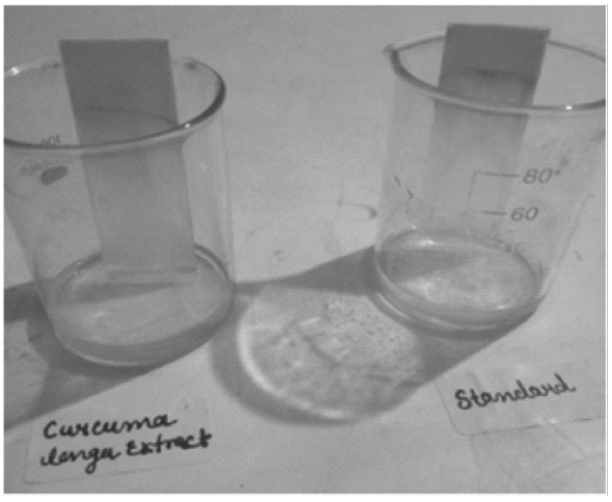



Fig. 1: TLC Plates




Fig. 2: Column chromatography of *Curcuma longa*

**Preformulation Studies**

The preformulation study was performed in order to assure the authenticity of sample drug and determination of some parameters for development of formulation.

Preparation of Calibration Curve

Table 4: Absorbance of curcumin at different concentrations

| S. No. | Concentration (?g/mL) | Absorbance |
|---|---|---|
| 1 | 1 | 0.361 |
| 2 | 2 | 0.538 |
| 3 | 3 | 0.725 |
| 4 | 4 | 0.964 |
| 5 | 5 | 1.348 |

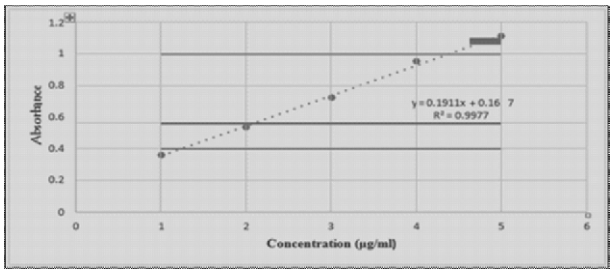



Fig. 3: Standard curve of Curcumin in ethanol

**UV analysis**

After scanning for λmax over a range of 300-500 nm on a double beam UV Spectrophotometer. The maximum absorbance of curcumin in ethanol was found to be at λmax 424 nm which was matched with reference indicated that the drug sample was pure.

Investigation of Physicochemical Characteristics of Curcumin:

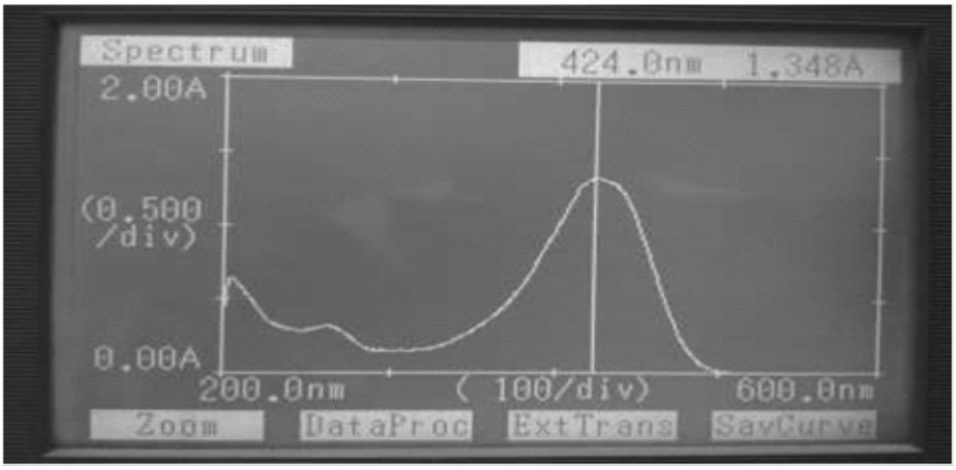



Fig. 4: Spectrum (λmax – 424nm) of curcumin in ethanol

**Organolepticcharacteristics**

Curcumin shows yellow in colour, pungent odor and bitter in taste

**Melting point:** Melting point of curcumin was found to be181±3°C were compared with standard 183°C

Evaluation parameters of transdermal patches

**Physical appearance:** Prepared transdermal patch was found to be yellowish color, having sufficient flexibility and smooth appearance on physicalevaluation.

**Thickness:** Thickness of patch was calculated by digital Vernier caliper. Thickness of FC3 was found to be 0.238±0.1087 mm, have low standard deviation value that ensures uniformity in thickness and distribution of polymer, drug and other excipients of formulations.

**Weight variation:** Weight of different patches of formulation (FC3) found to be 729.312±3.2113 mg, showed low standard deviationvalue.

**Percentage moisture loss:** It is similar to moisture content test, done to assess the physical stability of patches in dry condition. The percentage moisture loss patch was found to be 4.913±0.279 in first 24 h, reflecting low moistureloss.

Table 5: Moisture loss of formulation calculated as mean±SD

| S. No | Formulation Code | Moisture loss after 12 hrs. (%±SD) | Moisture loss after 24 hrs. (%±SD) | Moisture loss after 48 hrs. (%±SD) |
|---|---|---|---|---|
| 1 | FC3 | 2.261±0.185 | 4.913±0.279 | 6.213±0.159 |

**Water vapour permeation test**

The water vapor permeation for prepared patches have low, these results indicates that all the formulations are permeable to water vapor.

Table 6: Water vapour permeation through patch calculated as mean±SD

| S. NO. | Formulation code | WVP (gmm/cm$^2$) after 24 h | WVP (gmm/cm$^2$) after 48 h | WVP (gmm/cm$^2$) after 72 h |
|---|---|---|---|---|
| 1 | FC3 | 0.0071±0.0001 | 0.0215±0.000451 | 0.0359±0.0030 |

**Drug content**

The drug content was determined by spectrometric method. All formulation have showed that a near uniform drug content ranging from 83.0018±1.31% to 94.5172±0.73% and low standard deviations. This study revealed that the process employed to prepare the transdermal patches were capable of affording uniform drug distribution within formulations and uniform drugcontent.

Table 7: Drug content of prepared transdermal patches

| S. No | Formulation code | Drug content (%±SD) |
|---|---|---|
| 1 | FC1 | 93±0.92 |
| 2 | FC2 | 92±1.53 |
| 3 | FC3 | 93±1.14 |
| 4 | FC4 | 94±0.89 |
| 5 | FC5 | 95±0.56 |

*In-Vitro* Drug Release Studies

On the data obtained from *in-vitro* release formulation FC3 shows sustained and controlled release as well as have high release among all other formulations, therefore formulation FC3 were further evaluated for Physicochemical properties (such as thickness, weight variation, moisture content, moisture loss, moisture uptake, water vapour permeation, drug content, mechanical properties (Tensile strength and % elongation) and animal studies (Skin irritation,Anti-inflammatory).

Table 8: Cumulative % drug release of formulation (FC1 to FC3) which contains propylene glycol as permeation enhancer and Glycerol as plasticizer.

| S. No. | Time (h) | FC1 | FC2 | FC3 | FC4 | FC5 |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | 3.461±0.224 | 4.251±0.177 | 3.611±0.478 | 2.268±0.273 | 2.902±0.344 |
| 3 | 2 | 7.532±0.322 | 5.265±0.327 | 6.609±0.281 | 6.4844±0.778 | 4.919±0.495 |
| 4 | 3 | 11.369±0.537 | 7.914±0.328 | 9.951±0.521 | 10.252±0.465 | 6.320±0.275 |
| 5 | 4 | 14.651±0.350 | 11.219±0.507 | 12.403±0.858 | 13.658±0.428 | 7.255±0.189 |
| 6 | 5 | 16.256±0.657 | 13.999±0.907 | 15.259±0.186 | 15.273±0.692 | 8.915±0.377 |
| 7 | 6 | 18.282±0.348 | 16.510±0.356 | 17.460±0.357 | 16.993±0.613 | 9.232±0.346 |
| 8 | 7 | 21.232±0.306 | 18.620±0.642 | 19.920±0.540 | 17.213±0.467 | 10.065±0.790 |
| 9 | 8 | 22.549±0.389 | 21.231±0.644 | 22.246±0.438 | 21.417±0.713 | 13.382±0.594 |
| 10 | 9 | 24.756±0.411 | 23.529±0.924 | 23.842±0.365 | 22.243±0.475 | 17.424±0.200 |
| 11 | 10 | 27.265±0.640 | 24.936±0.698 | 25.236±0.335 | 25.173±0.326 | 22.363±0.629 |
| 12 | 11 | 32.659±0.502 | 26.518±0.429 | 27.737±0.775 | 27.104±0.424 | 25.923±0.208 |
| 13 | 12 | 38.235±0.730 | 30.741±0.719 | 30.497±0.881 | 29.571±0.661 | 26.613±0.258 |
| 14 | 24 | 64.526±0.436 | 45.841±0.668 | 47.969±0.419 | 40.455±0.385 | 32.614±0.218 |

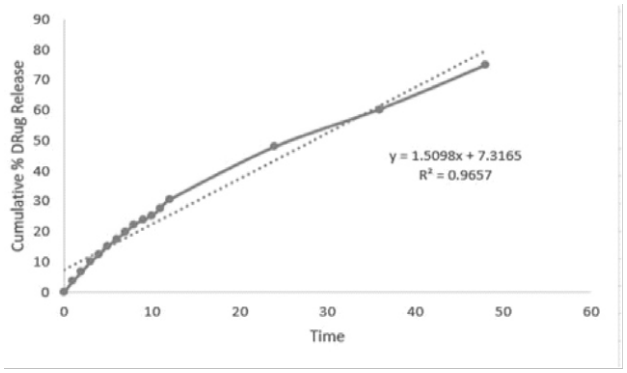



Fig. 5: Zero order release graph of formulation FC3 in Ethanol and PBS (50:50)

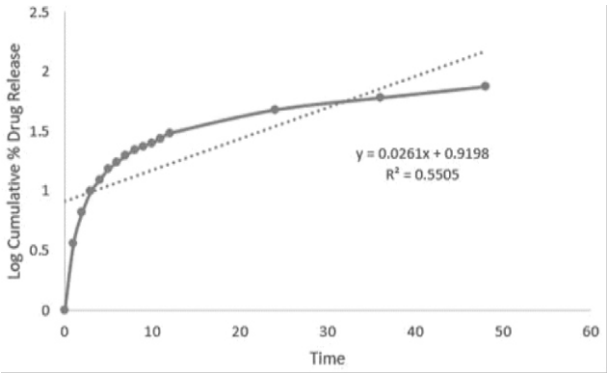



Fig. 6: First order release graph of Formulation FC3 in Ethanol and PBS (50:50)

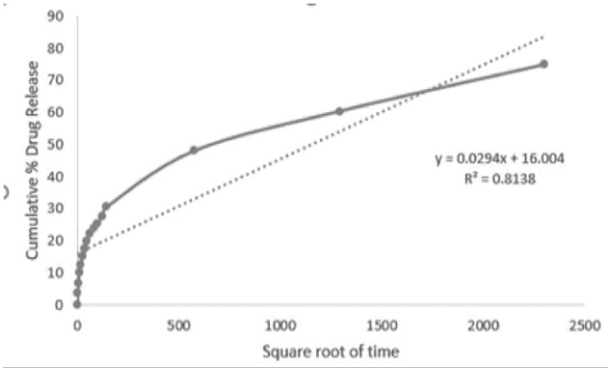



Fig. 7: Higuchi release graph of formulation FC3 in Ethanol and PBS (50:50)

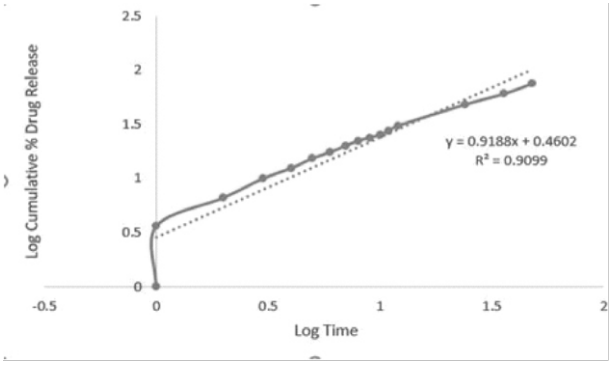



Fig. 8: Korsemeyer'sPeppas release graph of formulation FC3 in Ethanol and PBS (50:50)

5.6. *In vivo* Anti inflammatory Activity

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis.

Table 9: Anti inflammatory efficacy of transdermal patches using carrageen induced hind paw oedema in rats

| Group No. | Treatment | Time (min.) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 15 min | 45 min | 90 min | 60 min | 4 hr | 6 hr | 12 hr |
| I | Control | 2.02±0.260 | 2.35±0.091 | 2.46±0.129 | 2.54±0.143 | 2.56±0.167 | 2.68±0.140 | 2.84±0.122 |
| II | Standard (Indomethacin) | 2.00±0.167 | 2.15±0.113 | 1.62±0.178 | 1.11±0.071 | 1.00±0.085 | 0.79±0.172 | 0.59±0.145 |
| III | Curcumin (400 mg/kg) | 2.10±0.092 | 2.35±0.096 | 2.70±0.138 | 2.78±0.125 | 2.57±0.077 | 1.83±0.247 | 1.37±0.196 |
| IV | Formulation (20 mg) | 2.07±0.181 | 2.44±0.129 | 2.54±0.119 | 2.45±0.083 | 2.06±0.063 | 1.67±0.124 | 1.07±0.154 |
| V | Formulation (40 mg) | 2.03±0.072 | 2.22±0.126 | 2.43±0.130 | 2.46±0.093 | 2.03±0.131 | 1.58±0.260 | 0.91±0.182 |

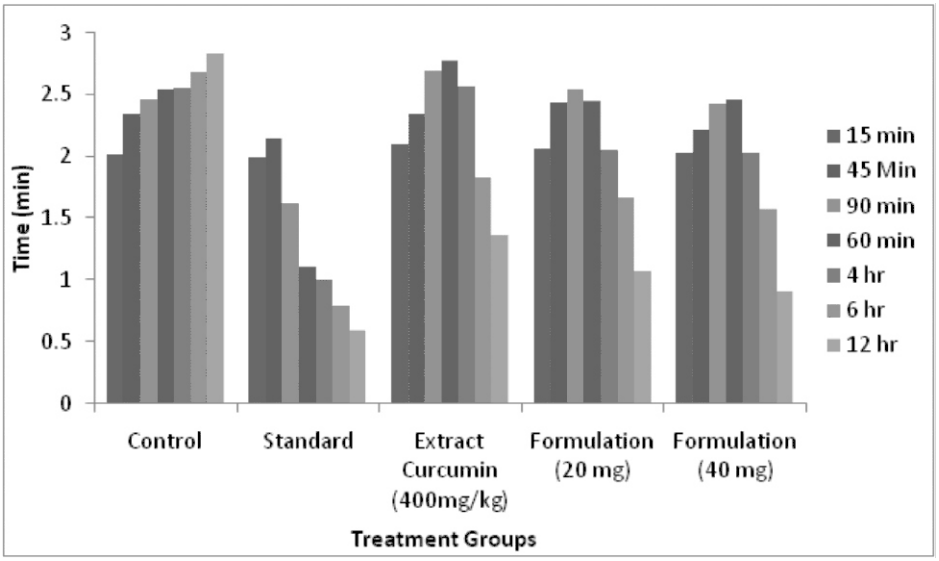



Fig. 9: Effect of anti inflammatory activity of transdermal patches using carrageen induced paw oedema technique.

## CONCLUSION

Suitable parameters for the phytochemical screening, physicochemical characterization, preformulation of transdermal patches, *in-vitro* drug release and *in-vivo* anti-inflammatory activities of formulated transdermal were evaluated. The anti-inflammatory activity decreased with increasing time in case of control (Indomethacine) group whereas the curcumin (400 mg/kg) shows maximum activity at maximum time (12 h) the activity increasing with increasing time intervals. Formulation with 40 mg/kg is better than formulation 20 mg/kg. Curmuminshowed maximum activity compared to other formulation and standard.

## REFERENCES

Afaq, F., Adhami, V.M., Ahmad, N. and Mukhtar, H. 2002. Botanical antioxidants for chemoprevention of photocarcinogenesis. *Front Biosci*. 7:d784-92.

Ahmad, I., Mehmood, Z. and Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J.Ethnopharmacol*.62(2):183-93.

Ajayi, I.A., Ajibade, O. and Oderinde, R.A. 2011. Preliminary Phytochemical Analysis of some Plant Seeds. *Res. J. Chem. S*.1(3): 58-62.

Ammon, H.P. and Wahl, M.A. 1991. Pharmacology of *Curcuma longa*. *Planta Med*. 57:1-7.

Ashraf, K., Mujeeb, M., Altaf, A., Ahmad, S., Amir, M. and Mallick, M.N. 2012. Validated HPTLC analysis method for quantification of variability in content of curcumin in *Curcuma longa* L (turmeric) collected from different geographical region of India. *Asian Pac. J. Trop Biomed*. 5:584-8.

Das, B. 1991. Materia Medica of Ayurveda. B Jain Publisher, New Delhi, 8.

Jagtap, A.G. and Karkera, S.G. 1999. Potential of the aqueous extract of *Terminalia chebula* as an anticaries agent. *J.Ethnopharmacol*. 68(1-3):299-306.

Jain, D.K., Nayak, A., Patel, P., Jain, S. and Khan, M.A. 2019. Appraisal of in vitro Antioxidant and *in vivo* Anti-Inflammatory Activities of Various Extracts from the Fruits of *Vitis vinifera* L. *Sch. Acad. J. Pharm*. 8(3): 86–93.

Jain, D.K., Patel, N.S., Nagar, H., Patel, A. and Chandel, H.S. 2012. Anti-arthritic activity of tridax procumbens ethanolic extract of leaves. *J. Pharm. Sci*.2(4): 80-86.

Kokate, C.K., Purohit, A.P. and Gokhale, S.B. 1993. Pharmacognosy NiraliPrakashan Pune edition 45$^{th}$p.g. 7.20-32.

Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P. and Mukherjee, P.K. 2007. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. *Int. J. Pharm*. 330:155-63.

Priyadarsini, K.I. 2014. The chemistry of curcumin: From extraction to therapeutic agent. *Molecules,* 19:20091-112.

Purseglove, J.W., Brown, E.G., Green, C.L. and Robbins, S.R. 1981. Spices. Vol. 1. London, New York: Longman.

Rana, M., Maurya, P., Reddy, S.S., Singh, V., Ahmad, H. and Dwivedi, A.K. 2016. A standardized chemically modified *Curcuma longa* extract modulates IRAK-MAPK signaling in inflammation and potentiates cytotoxicity. *Front. Pharmacol*. 7:223.

Sato, Y., Oketani, H., Singyouchi, K., Ohtsubo, T., Kihara, M., Shibata, H. and Higuti, T. 1997. Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* RETS. against methicillin-resistant Staphylococcus aureus. *Biol. Pharm. Bull*. 20(4):401-4.

Srikanthmurthy, K.R. 2000. Bhavaprakasha of Bhavamishr. (Krishnanda Academy, Varanasi), 160.

The Unani pharmacopeia of India. 2010. Department of AYUSH, Ministry of health and family welfare, Government of India.

Varrier, P.S. 1996. Indian Medicinal Plants: A Compendium of 500 species. Edited by PK Warrier, VPK Nambiar and C Ramankutty. Vol. 5. Hyderabad: Orient Longman. P, 263.

Vavilova, N.I. 1990. Turmeric a new spice and medicinal plant. *Skogo Inst. Rastenievodstvalm*. 4:77-9.